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The purpose of this study was to examine the association of demographic factors, serum Vitamin D levels, hypertension (HTN) (by HTN diagnosis, systolic blood pressure [SBP] and diastolic blood pressure [DBP]), serum (hs-CRP) and endothelial measures of inflammation upon the prevalence of depression in adults with coronary artery disease (CAD) from central North Carolina. A literature derived theory, the Puglisi Model of Vitamin D Levels' Associations with Depression, guided this study. Vitamin D levels, measures of blood pressure, and serum and endothelial measures of inflammation, were theorized as being associated with depression.

A cross-sectional, associational design was employed in the parent study from which previously frozen alliquoted blood of subjects with CAD was further analyzed to assess the serum Vitamin D levels and liver function. This convenience sample of 101 persons with CAD who presented between 2007 and 2010 at the University of North Carolina Hospital's Cardiac Catheterization lab was utilized. The majority of the sample of well-controlled persons with CAD was male (66%), White (81%), had hypertension (81%), and low serum Vitamin D levels (82%). Depression, found as a diagnosis in 27%, was not significantly associated with Vitamin D levels ($p = 0.17$), even when controlling for demographic factors (AOR 0.96; $p = 0.13$; 95% CI [.90 – 1.01]). There were no differences between brachial artery flow mediated dilation (BAFMD), augmentation index, and high sensitivity C-reactive protein (hs-CRP) by depression group, but there was for reactive hyperemia index (RHI) [$t = 1.97$; $df = 99$; $p = 0.05$]. Vitamin D levels were inversely associated with both SBP ($p < 0.001$) and DBP ($p < 0.001$), but Vitamin D levels were not associated with a diagnosis of HTN (AOR 0.97; $p = 0.28$; 95% CI [.92, 1.02]. Controlling for the potential confounders of age, sex, race, body mass index, liver and kidney functions did not alter the significant association between Vitamin D levels and SBP and DBP

($p = 0.05$). Vitamin D levels were significantly associated with two inflammatory measures—hs-CRP and augmentation index, but not with BAFMD and RHI. When controlling for age, sex, race, BMI and Vitamin D levels, only hs-CRP but none of the three endothelial measures of inflammation (RHI, BAFMD nor augmentation index [AI]), were associated with depression (AOR 0.956; $p = 0.13$; 95% CI [.90, 1.01]).

Nurses should be aware that most of the adults with CAD herein had low or insufficient Vitamin D levels, and that Vitamin D levels may significantly affect SBP and DBP in persons with CAD and perhaps other populations as well. Many persons have depression around the time of their cardiac event or thereafter. Because increased morbidity and mortality occur in individuals with decreased Vitamin D levels, and depression, even when identified and treated in persons with CAD is associated with worsened outcomes, appropriate screening for and treatment of low serum Vitamin D levels is needed. Thus, advanced practice clinicians caring for persons with CAD should encourage screening of Vitamin D levels, and treatment of low levels with appropriate supplementation.

Further studies are needed to explore why some endothelial measures are associated with Vitamin D levels and depression, and others are not. Additional studies should seek to confirm the inverse association of Vitamin D levels with SBP and DBP while accounting for season of the year and other potential confounders. Finally, studies should utilize a depression screening tool to test the Puglisi model's proposed association between low Vitamin D levels with an increased occurrence of depression in both persons with CAD and other populations.

THE ASSOCIATION OF VITAMIN D LEVELS, BLOOD PRESSURE, INFLAMMATION AND DEPRESSION IN PERSONS WITH CORONARY ARTERY DISEASE

by

Janis Panzenhagen Puglisi

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Approved by

_____
Committee Chair

To Sasha, Ernie, Mom, Dad and all of my Heavenly Father's angels on earth:

thank you and I love you each so very much!

APPROVAL PAGE

This dissertation written by JANIS PANZENHAGEN PUGLISI has been approved by the following committee of the Faculty of The Graduate School at The University of North Carolina at Greensboro.

Committee Chair

_____
Patricia B. Crane

Committee Members

_____
Susan Letvak

_____
Elizabeth Van Horn

_____
Laurie Wideman

_____
Craig Lee

_____
Date of Acceptance by Committee

_____
Date of Final Oral Examination

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CHAPTER I

BACKGROUND

Introduction

Coronary artery disease (CAD) is the leading cause of death and disability in the United States (Hoyert & Xu, 2012). Indeed, CAD affects over a quarter of the population; approximately 7% of adults aged 45-64, and approximately 20% of adults aged 65 years and older have CAD (Centers for Disease Control and Prevention [CDC], 2012). While age-adjusted prevalence of CAD has declined from 6.7% to 6.0% between 2006 and 2010 due to improved treatments and more control of risk factors, mortality from CAD still occurs in 25% (CDC, 2012; Hoyert & Xu, 2012). Nearly half of Americans have one of three common risk factors for CAD: hypertension (HTN), increased levels of low density lipoprotein cholesterol, and smoking (CDC, 2012). Of these three risk factors, HTN is present in 33% of adults in the United States (Writing Group for the American Heart Association, 2013). The high prevalence of HTN is a concern because 7% of adults have undiagnosed HTN, only 78% are taking medication for their HTN, and just 65% have their HTN controlled (Writing Group for the American Heart Association, 2013). More concerning is that HTN is present in 69% of those having a first myocardial infarction or 77% of those having a stroke (Heart Disease and Stroke, 2013). Thus, a large number of undiagnosed and sub-optimally managed persons with HTN are at risk for CAD and health events or conditions associated with HTN and CAD.

While CAD is frequently associated with HTN, CAD is also associated with depression. In fact, depression has been declared an independent risk factor for the development of CAD (Pozuelo et al., 2009). It remains unclear, however, the exact mechanism of the association of

depression in CAD: is depression a consequence or a cause of CAD (de Jonge & Roest, 2012; Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Investigators et al., 2003; Mendes de Leon et al., 1998)? Khawaja, Westermeyer, Gajwani and Feinstein (2009) conducted a comprehensive review of the literature on the association of depression and CAD and determined that the relationship between CAD and depression is likely bidirectional. Because depressed persons are more likely to have both a fatal and non-fatal myocardial infarction or angina as compared to non-depressed persons, this makes understanding and controlling the mechanisms of depression in CAD important to improving health outcomes (American College of Cardiology Foundation et al., 2012; Saran, Puri, & Agarwal, 2012). More concerning is that even when depression is present, identified and treated, improvement in depression has not been shown to improve cardiovascular outcomes (American College of Cardiology Foundation, et al., 2012; Glassman et al., 2002; Writing Committee for the ENRICHD Investigators, 2003). Therefore, further research to explore the nature of the relationships between depression, CAD, and HTN is important to gain an understanding of these relationships and subsequently to develop interventions that will positively affect outcomes by minimizing risk.

Background and Significance

Depression statistics indicate this condition has now become the world's leading cause of disability (World Health Organization, 2012). Depression is present in 6.7% of the adult population of the United States during any 12-month timeframe (National Institutes of Mental Health, n.d.). This is a significant concern because depression, like CAD, affects quality of life (American Heart Association Statistics Committee and Stroke Statistics Committee et al., 2012; Pozuelo, et al., 2009; Renwick et al., 2012), lowers productivity (Khawaja, Westermeyer, Gajwani, & Feinstein, 2009; Stewart, Ricci, Chee, Hahn, & Morganstein, 2003), and leads to poorer health outcomes (Wing, Phelan, & Tate, 2002). Depression may contribute to memory

and cognitive deficits that reduce executive function and, therefore, lessen the ability to take medications correctly and negatively influence health outcomes (DiMatteo, Lepper, & Croghan, 2000; Goldston & Baillie, 2008). Depression also leads to persons not fully complying with lifestyle management practices, as well as a lack of follow-through on further testing or follow-up treatment (American College of Cardiology Foundation, et al., 2012; DiMatteo, et al., 2000; Eze-Nliam, Thomgs, Lima, Smith, & Ziegelstein, 2010).

Multiple physiologic mechanisms exist to increase the incidence of depression, including decreased cerebrovascular perfusion, suboptimal therapy, and even low cardiovascular fitness (Aberg et al., 2012). Swedish males, aged 18 years, were followed for 3-40 years (Aberg et al., 2012). The researchers found that lower fitness at age 18 was associated with a later increased incidence of serious depression in adulthood (Aberg et al., 2012; de Jonge & Roest, 2012). Because of a lack of certainty of the pathophysiologic mechanisms linking depression to CAD and cardiovascular diseases, there remains a lack of clearly targeted interventions, including pharmacotherapies, to address the physiologic dysregulation that results in depression (Joynt & O'Connor, 2005). Thus, understanding HTN in relation to depression is important.

Depression is a known risk for CAD, and there is literature suggesting that HTN may be independently related to depression (Meng, Chen, Yang, Zheng, & Hui, 2012; Scalco, Scalco, Azul, & Neto, 2005). Hypertension is a risk factor for both coronary artery and cerebrovascular diseases, possibly because of the effect of HTN upon the brain wherein a decrease in cerebrovascular perfusion leads to neuronal injury. Indeed, the brain and body are linked with a stress response which results in physiological changes such as disturbed platelet function, increased heart rate, inflammation, as well as sympathetic and hypothalamic-pituitary axis stimulation (de Jonge & Roest, 2012; Michelson, 2009; Scalco et al., 2005). Because of the brain

and body's correlational response, it is plausible that physiologic changes could contribute to the pathogenesis of depression.

Multiple physiological mechanisms may contribute to HTN and depression. One mechanism resulting in HTN occurs when an increased amount of Angiotensin II (Ang II) is produced peripherally and within the brain. Angiotensin II results in potent vasoconstriction. Atherosclerosis also narrows the arterial lumen. Consequently, HTN that is coupled with atherosclerosis results in diminished cerebrovascular flow which extends any neuronal injury previously initiated by atherosclerosis (Saavedra, 2012) and potentially leading to mood disorders, including depression. Thus, both HTN and atherosclerosis are mechanisms of coronary and peripheral arterial disease wherein neuronal injury occurs, and these conditions lead towards systemic inflammation (National Heart, Lung, and Blood Institute, 2011).

Another mechanism for an association between HTN and depression is that persons with HTN usually have atherosclerosis, which is associated with inflammation (Writing Group for the American Heart Association, 2013). Hypertension results in damage to the endothelial lining of the arterial vasculature and ensues when the endothelium becomes injured and dysfunctional which may influence inflammation (Cushman, 2003). The endothelium is dynamic tissue that secretes various chemicals (nitric oxide, prostacyclin and endothelin) to regulate blood pressure, coagulation and platelet activity (Tousoulis, Charakida, & Stefanadis, 2005). Nitric oxide causes potent vasodilation. Endothelial dysfunction is a loss of endothelium-dependent vasodilation. Endothelial dysfunction is associated with HTN, present in the development of atherosclerosis, related to plaque buildup, and is proportionally related to an increased incidence of cardiovascular events (Gonzalez & Selwyn, 2003; Tousoulis, et al., 2005).

Endothelial dysfunction triggers an inflammatory cascade (Endemann & Schiffrin, 2004). This inflammation involves the increased production of cytokines, acute phase proteins (C-

reactive protein [CRP], plasminogen activator inhibitor-1 [PAI-1]) and chemokines. Cytokines are proteins produced by cells that modulate the body's reaction to disease, infection and injury. Certain cytokines such as Interleukin-1 (IL-1) and Interleukin-6 (IL-6) initiate the body's inflammatory response by increasing platelets, white blood cells, and acute phase proteins, such as C-reactive protein (CRP) and fibrinogen (Alonso-Martinez et al., 2002; Ershler & Keller, 2000). Both CRP, IL-1, and IL-6 have been associated with depression (Howren, Lamkin, & Suls, 2009; Kop et al., 2002; Ma et al., 2011). In fact, in an older population without evidence of myocardial ischemia, both CRP and fibrinogen were positively associated with depression (Kop, et al., 2002). Even mere aging results in a chronic low-grade inflammatory response perhaps due to unregulated excess cytokine production of IL-6 and CRP (Wilkerson & Sane, 2002). Increased inflammation occurring with age is a significant concern because HTN and CAD also increase with age. Therefore, an increase in inflammation may compound the development of CAD and HTN affecting the older adult's general health and perhaps even their mood.

Inflammatory markers have been found to be higher in those who are depressed and in those with subclinical depression (Howren, et al., 2009). In female adolescents, a high sensitivity C-reactive protein (hs-CRP) level may exist 6 months later even if depressive symptoms have abated (G. E. Miller & Cole, 2012). Mood has even been found to be worse in those with prior cardiovascular events (Peters et al., 2010). Studies have suggested that if depressed mood precedes cardiovascular disease or mortality, this occurrence is due to the behavior of the depressed person who exercises less, engages in less prevention, or possibly negative effects from taking antidepressant drugs (Peters et al., 2010). Other pathways explaining how depression may lead to poor cardiovascular outcomes include HTN, endothelial injury, atherosclerosis or thrombus formation in those not adhering to lifestyle or suggested therapies (Peters, et al., 2010). Despite these pathways, the mechanism limiting HTN and endothelial dysfunction is important

for preventing poor coronary outcomes and depression. Clearly, the relationship of depression and cardiovascular disease is extremely complex (de Jonge & Roest, 2012; Peters, et al., 2010).

A possible novel mechanism beyond HTN, atherosclerosis, endothelial dysfunction and inflammation, which might influence depression is the physiological cascade associated with low serum Vitamin D levels. Vitamin D receptors (VDRs) are in abundance within the brain. While it is unclear if any of the functions of Vitamin D in the brain may be directly related to major depression, many areas in the brain rich in VDRs have been shown to have abundant enzyme activity for metabolizing pre-Vitamin D into active Vitamin D, or calcitriol (Bertrone-Johnson, 2009). Perhaps for this reason, low serum Vitamin D has been associated with depression in a cardiovascular population (May et al., 2010). What is not understood is if lower serum Vitamin D level is a cause or consequence of depression (Berk et al., 2007; Bertrone-Johnson, 2009; Parker & Brotchie, 2011). Despite the lack of clarity of the direction of the relationship, Vitamin D levels have been associated with depression in multiple populations of varying ages and health conditions (Ganji, Milone, Cody, McCarty, & Wang, 2010; Jorde, Sneve, Figenschau, Svartberg, & Waterloos, 2008; Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Investigators, et al., 2003; May, et al., 2010; Stewart & Hirani, 2010).

In summary, depression is a serious, highly prevalent disease that results in decreased quality of life, poor health outcomes and may limit personal achievement. Hypertension, endothelial dysfunction, and inflammation are also highly prevalent and may contribute to depression via neuronal injury within the brain. Low Vitamin D levels are common in our modern world in which many work inside for long hours and are encouraged to avoid the sun or use sunscreen. Currently, we do not understand fully how the traditional and the newer cardiovascular risk factors such as serum Vitamin D and hs-CRP levels contribute to depression. We do know that suboptimal Vitamin D levels are associated with increased cardiovascular

morbidity (Fiscella & Franks, 2010). Because of the morbidity and mortality associated with HTN, CAD and depression, all factors that may influence the occurrence and severity of depression must be understood to enhance patient outcomes and lower our nation's burgeoning health costs. As a result, this study was conducted to examine the relationship of depression, the number one cause of disability worldwide, along with Vitamin D levels, HTN, and serum and endothelial measures of inflammation (World Health Organization, 2012).

Depression

Depression is present in 6.7% of the adult population in the United States during any 12-month time frame (National Institutes of Mental Health, n.d.). Thus in 2010, nearly 16 million of the 235 million adults in the United States experienced a depression episode (United States Census Bureau, 2011). At best, the relationship between depression and health outcomes is complex. Depression remains a highly prevalent, burdensome disease that is recurrent and affects productivity and health outcomes (Black, Markides, & Ray, 2003; Miller et al., 2007). For instance, depression occurring in adolescents is known to be a risk factor for poorer self-rated health, higher work impairment in later life and the need for more health care utilization (Cha, Patel, Hains, & Mahan, 2011; D. K. Miller, Constance, & Brennan, 2007). Depression is also linked to poor health outcomes in many populations, including diabetics, wherein the depressed have higher glycosylated hemoglobins (Wing, et al., 2002), and in post stroke individuals, who when depressed, experience more difficulty with mobility and general physical functioning than in those who are not depressed (Goodwin & Devanand, 2008**)**. In Mexican American diabetics, the synergistic interaction between diabetes and depression is suspected to have an unknown physiologic link, with the depressed diabetics having worse health outcomes, including more macro and microvascular complications and disability than those without depression (Black, Markides & Ray 2013). Thus, depression negatively impacts the lives of those affected and their

families in multiples ways, including increased morbidity and poorer health outcomes (Black, et al., 2003; Coventry & Gellatly, 2008; Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Investigators, et al., 2003; Michelson, 2009; Writing Committee for the ENRICHD Investigators, 2003).

Despite controlling for chronic medical disorders, persons with depression continue to report increased medical symptoms (Pozuelo, et al., 2009). The high prevalence of depression affects families and society by its relationship with decreased quality of life and productivity (Bansil et al., 2010; Ndokera & MacArthur, 2010). For instance, women's mood affects the health and outcomes of both their fetus and infant (Bansil, et al., 2010; Ndokera & MacArthur, 2010). Quality of life is also predicted by depressive symptoms (Renwick, et al., 2012). Quality of life can also be affected by treatment non-adherence that is characteristic in those with depression; in fact, persons who are depressed with cardiac disease are less likely to follow through with diet, lifestyle, exercise, testing and follow-up recommendations (American College of Cardiology Foundation, et al., 2012; DiMatteo, et al., 2000; Pozuelo, et al., 2009). Additionally, those who are depressed are two to four times more likely to be non-adherent to recommended therapies which for persons with CAD would include disease stabilizing anti-hypertensives, aspirin, and statin cholesterol drugs (American College of Cardiology Foundation, et al., 2012; DiMatteo, et al., 2000). For example, 64% of those with newly diagnosed depression do not fill anti-depressants within 90 days (Cantrell, Priest, Cook, Fincham, & Burch, 2011). Because the depressed exhibit memory and cognitive deficits that may reduce executive function and the ability to take medications correctly, depressed persons with HTN have been found to not follow the prescribed regimen of care the more depressed they were (Goldston & Baillie, 2008). Yet, it is unclear what variables other than depression may affect adherence with medication because age, religion, comorbidities, number of medications, income, and family

history of HTN did not differ in a recent study of non-depressed Black women's adherence to anti-hypertensive medications (Abel, 2011). Clearly, further research is needed to identify the variables associated with depression.

The Association of Depression and Hypertension

Depression is linked to HTN, but the literature has been inconsistent about how the two are associated (Hildrum, Romild, & Holmen, 2011; Meng, et al., 2012). One theory explaining the relationship is that emotional stress might increase cortisol or catecholamine release, and these tend to increase both the heart rate and blood pressure (Fraser et al., 1999). Grewen, Girdler, Hinderliter and Light (2004) found that higher depression symptom scores were significantly associated with higher ambulatory blood pressures, but only in those with a family history of HTN. In contrast, a recent meta-analysis of prospective cohort studies confirmed that depression is likely an independent risk factor for the development of HTN with depression, with those who are depressed having a 42% increased risk (RR=1.42; 95% CI [1.09 – 1.86]; $p = 0.009$) of developing HTN (Meng, et al., 2012).

Like depression, HTN is a complex disease associated with poorer health outcomes due to an associated increased morbidity and mortality. There is a relative risk of 2.3 – 2.7 for stroke and possibly cardiovascular related deaths in elderly persons with increased numbers of depressive symptoms and HTN (Simonsick, Wallace, Blazer, & Berkman, 1995). While HTN may be linked to depression, the literature is unclear about the populations at risk for having both conditions and the cause of the association (Hildrum, et al., 2011; Meng, et al., 2012). Hypertension and depression may be associated due to either environmental stress or genetics initiating the conditions (Fraser, et al., 1999; Grewen, Girdler, Hinderliter, & Light, 2004). In fact, it has been found that integrating treatment interventions for depression and HTN improves patient outcomes (Bogner & de Vries, 2008). Consequently, because HTN injures fragile

cerebral neurons beyond the altered function already present in depression, having HTN concurrently with depression may augment neuronal injury and dysfunction. Together, HTN and depression may also worsen health outcomes because of an associated decreased executive function and treatment adherence in those with both conditions (DiMatteo, et al., 2000; Goldston & Baillie, 2008; Khawaja, et al., 2009).

Endothelial Dysfunction

Endothelial dysfunction is an indirect measure of inflammation because the condition reflects inappropriate vasodilation and a pro-inflammatory state (Endemann & Schiffrin, 2004). The endothelium manifests as dysfunctional when there is ineffective or decreased vasodilatation, increased inflammation, or when it is contributing to a pro-thrombotic state (Endemann & Schiffrin, 2004). Loss of endothelium-dependent vasodilation is a typical feature present in the development of atherosclerosis and is related to future cardiovascular risk (Gonzalez & Selwyn, 2003). Endothelial dysfunction is also associated with HTN (Endemann & Schiffrin, 2004) and is proportionally related to an increased incidence of cardiovascular events (Tousoulis, et al., 2005). Not surprisingly, it is important to prevent endothelial dysfunction because the condition precedes the development of clinical cardiovascular disease by several years (Al Mheid et al., 2011).

Depression and Inflammation

Depression may be associated with inflammation as measured by hs-CRP levels (Howren, et al., 2009; Kop, et al., 2002; Ma, et al., 2011; Miller & Cole, 2012). Three proposed relationships between depression and inflammation exist: (a) depression leading to inflammation; (b) inflammation leading to depression, and (c) a bidirectional relationship between the variables (Howren, et al., 2009). Depression has been significantly associated with increased levels of hs-CRP in an elderly sample of those more than 65 years of age (Kop, et al., 2002). In fact, a meta-analysis of 51 studies from 1967 to January 2008 found a highly significant positive correlation

between increased hs-CRP and depression scores ($d = 0.22$, $p < 0.001$) (Howren et al., 2009). Therefore, evidence supports a relationship between inflammation, specifically hs-CRP, and depression (Howren, et al., 2009).

Depression is also associated with atherogenesis which involves inflammatory cytokines (Pearson et al., 2003). Depression may be further related to endothelial dysfunction, which is a marker of inflammation (American College of Cardiology Foundation, et al., 2012). As a result of the multiple associations between HTN, endothelial dysfunction, inflammation and depression, a study of the inflammatory factors associated with depression will add to the science and possibly help clinicians to identify not only those who are at risk for depression, but to understand those also at risk for HTN, endothelial dysfunction and subsequent CAD.

Depression and Vitamin D Levels

A novel contributor to depression is Vitamin D levels (Ganji, et al., 2010; Jorde, et al., 2008). Vitamin D is an essential fat-soluble vitamin obtained from dietary sources, vitamin supplementation or skin exposure to sunlight. Serum Vitamin D deficiency is enhanced by a limitation of time in sunlight, the season of the year, reduced daylight, the use of sunscreen, darker skin pigmentation, clothing choice, aging, and a lack of supplementation or dietary intake of Vitamin D food sources. Deficiency is common: More than 50% of Latino and Black adolescents in Boston (Holick et al., 2011) and more than 1 billion people world-wide are Vitamin D deficient (Zhang et al., 2012).

There are a plethora of Vitamin D receptors within the brain. It is unclear if any of the functions of Vitamin D in the brain may be related to depression, although many areas rich in receptors have been shown to have abundant enzyme activity for metabolizing pre-Vitamin D into calcitriol (Bertrone-Johnson, 2009). A limited number of randomized controlled trials (RCTs) and other emerging evidence denote the association of Vitamin D levels with depression

in multiple populations of varying ages and health conditions (Ganji, et al., 2010; Jorde, et al., 2008; Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Investigators, et al., 2003; May, et al., 2010). Additionally, after one year a double blinded RCT of treatment with Vitamin D in overweight or obese subjects revealed significant improvement of depression scores (Jorde, et al., 2008).

Low Vitamin D levels may be associated with indirect pathways leading to depression. The first such pathway occurs when low serum Vitamin D contributes to increased blood pressure due to an up-regulated renin-angiotensin system (RAS) (Li et al., 2004), and the resultant altered cerebral perfusion from HTN leads to neuronal injury and mood change (Meng, et al., 2012). The second pathway by which low Vitamin D may lead to depression is through an increase in inflammation. Cytokines**,** such as hs-CRP, as well as endothelial dysfunction, reflect the body's state of inflammation which is associated with neuronal injury and possibly subsequent depression (Saavedra, 2012; Saavedra, Sanchez-Lemus, & Benicky, 2011).

In summary, Vitamin D levels may have several non-classical roles beyond their known effects on bone and for preventing falls (Holick, 2007; Sato, Iwamoto, Kanoko, & Satoh, 2005; Armin Zitterman & Gummert, 2010). These effects include roles in the regulation of mood, inflammation, and possibly blood pressure due to indirect effects by an influence upon endothelial function and inflammation (Jablonski, Chonchol, Pierce, Walker, & Seals, 2010). Vitamin D levels have already been found to be an independent risk factor for all-cause mortality (Zitterman & Gummert, 2010). However, a recent systematic review and meta-analysis was unable to demonstrate an effect of Vitamin D upon reduction in mortality or cardiovascular risk and concluded that current evidence is of low to moderate quality (Elamin et al., 2011). Hence, because depression is the world's number one cause of disability (Bromet et al., 2011) and CAD is related to HTN , inflammation, and endothelial dysfunction, it is imperative that potential

contributors to HTN, CAD and depression be understood (Murphy, Xu and Kochanek, 2012). Consequently, a study examining how Vitamin D levels, HTN, and inflammation are associated with depression is timely and contributes to filling an important public health need by potentially improving the knowledge of clinicians to appropriately intervene and positively affect outcomes.

Purpose

The purpose of this study was to examine the association of demographic factors, serum Vitamin D levels, HTN (by HTN diagnosis, systolic blood pressure [SBP] and diastolic blood pressure [DBP]), serum (hs-CRP) and endothelial measures of inflammation upon the prevalence of depression in adults with CAD from central North Carolina.

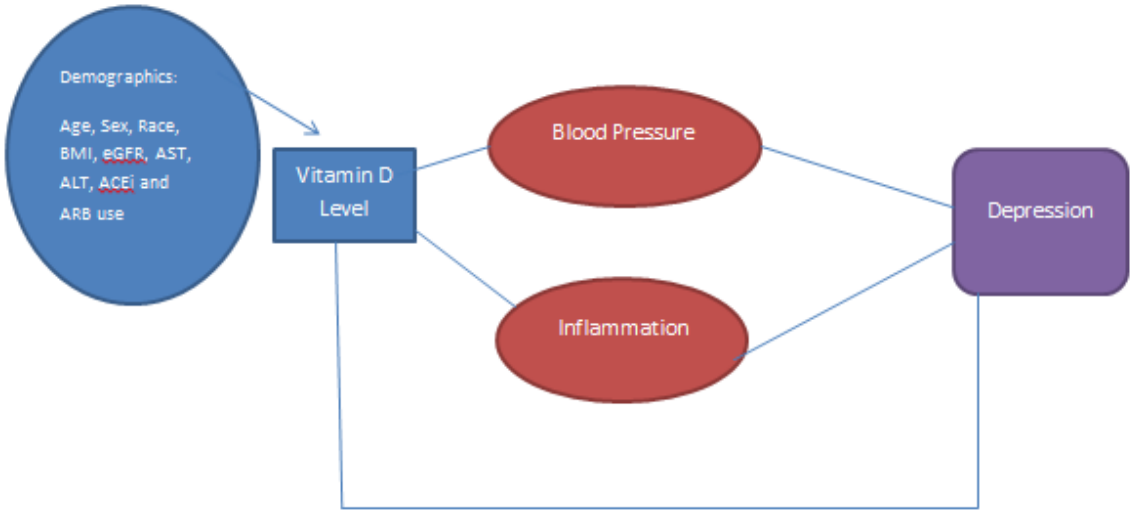
Conceptual Framework

The conceptual framework was derived from a review of the literature based upon the relationship between serum Vitamin D levels, HTN, inflammation and depression and is displayed in Figure 1. The model depicting Vitamin D levels depicts both direct and indirect association of serum Vitamin D levels upon depression.

Low serum Vitamin D levels have an established direct association with depression and this direct effect may be due to the abundance of Vitamin D receptors in the brain (Ganji, et al., 2010; Jorde, et al., 2008; Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Investigators, et al., 2003; May, et al., 2010). Because liver function and renal function can affect the synthesis of Vitamin D within the body, these were examined in separate analyses and placed within the model (Al-Badr & Martin, 2008; Jablonski et al., 2013; Putz-Bankuti et al., 2012; Vaidya & Williams, 2012).

Figure 1

The Puglisi Model of Vitamin D Levels' Associations with Depression



Note: BMI = body mass index; eGFR = estimated glomerular filtration rate by Modification of Diet in Renal Disease equation; AST = aspartate aminotransferase; ALT = alanine aminotransferase; ACEi = Angiotensin converting enzyme inhibitor; ARB = Angiotensin receptor blocker

Low serum Vitamin D levels also have an indirect association with depression, through pathways of serum and endothelial inflammation and blood pressure. Low serum Vitamin D levels have been associated with endothelial dysfunction in middle and older aged persons (Jablonski, et al., 2010). Additionally, low serum Vitamin D levels have an established association with depression in multiple populations (Forman et al., 2007; Ganji, et al., 2010; A. K. Gupta, Brashear, & Johnson, 2011; May, et al., 2010; Stewart & Hirani, 2010). This conceptual model describes an indirect association of serum Vitamin D levels upon HTN which may serve to initiate endothelial injury which would further enhance an inflammatory cascade (Endemann & Schiffrin, 2004). Thus, it is hypothesized that low serum Vitamin D levels may have both a direct association with the prevalence of depression, as well as an indirect relationship by influencing HTN and inflammation to augment the prevalence of depression.

Definitions

The following terms are defined to explicate the purpose of this study:

1. Age is the chronological length of life expressed in years. For this study age is recorded as a whole number representing the subject's lifespan in years since birth rounded down as was recorded in the primary data set.
2. Sex refers to a person's biological status that is typically categorized based upon sex chromosomes and gonads, and is usually male, female or intersex (when atypical combinations of features exist; American Psychological Association, 2011). In the primary data set, sex is recorded as only male or female.
3. Race refers to the self-identification of a person with one of the following categories according to the Office of Management and Budget: Caucasian (White), Black or African American, Asian, Native Hawaiian or Other Pacific Islander, or Alaska Native, which includes Central and South American Indians (Executive Office of the President, Office of Management and Budget (OMB), Office of Information and Regulatory Affairs, n.d.; U. S. Department of Health and Human Services, Office of Minority Health, 2010). In the primary dataset, race was recorded from the medical record and was recorded as only White, Black or Other.
4. Body mass index (BMI) is a measure of body fatness but does not measure fat directly, but correlates to underwater weighing and dual energy x-ray measures, making it an alternative for these other direct measures of fat (CDC, 2011a). Body mass index is operationalized in the primary dataset as a physiologic measure calculated in the uniform manner: weight (lb) /[height (in)]^2 x 703 (CDC, 2011a).
5. Liver function is clinically measured with great frequency by aspartate aminotransferase (AST) and alanine aminotransferase (ALT) analyses. An ALT test

measures an enzyme that is primarily in the liver, and to a lesser extent in the kidney, and changes in ALT are much more suggestive of liver disease than changes in AST. Aspartate aminotransferase occurs within many bodily tissues (heart, skeletal muscle, kidney, liver and others) and is an enzyme measured to detect and monitor liver damage, being more sensitive but less specific than ALT (Bakerman & Strausbauch, 2002). The normal laboratory values are as follows: AST > 40 IU/L, and ALT > 44 IU/L (Bakerman & Strausbauch, 2002).

6. Renal function in this study is the estimated glomerular filtration rate by the Modification of Diet in Renal Disease (MDRD) equation. The MDRD is considered to be a more accurate reflection of kidney function than the serum creatinine, and a more accurate calculation of the true GFR than that provided by the Cockcroft-Gault formula because age, change in muscle mass, chronic illness, poor nutrition and use of steroids can mask changes in the glomerular filtration rate (Fauci et al., 2008). In this study, renal function was defined as an abnormal eGFR, which in this study is less than 90 mLs/min (The Renal Association, n.d.).
7. Vitamin D is a fat soluble vitamin with numerous physiologic effects throughout the body, due to the plethora of VDRs in multiple tissues that include the vascular endothelium, cardiomyocytes and the brain (Zitterman, 2006). In this study, serum Vitamin D levels were operationalized as the level of 25(OH)D measured in ng/mL, as obtained from the analysis of frozen serum collected in the primary study, which was measured by Laboratory Corporation of America, hereafter Labcorp (Burlington, NC).
8. Hypertension is an abnormally elevated blood pressure wherein SBP represents the amount of force pushing against the arterial wall when the heart is contracting (the

numerator), and DBP is the amount of force on the arterial wall when the heart is at rest (the denominator). Hypertension is measured by these two blood pressure readings recorded in millimeters of mercury (mm Hg), with a blood pressure of more than 140/90 mmHg being considered HTN (The Joint National Committee on Prevention, 2004). Blood pressures were collected once using a Dinamap (GE Healthcare) after the subject laid supine for approximately 5 minutes. In the primary dataset, both SBP and DBP were recorded as numeric values with means and standard deviations, but HTN was recorded as present or not present based on a medical diagnosis in the medical record.

9. Inflammation is a nonspecific, compensatory physiologic response to cellular injury which is characterized as a protective physiological event that often involves leukocyte infiltration, redness, heat, pain, swelling, and loss of function of the inflamed tissue (Merriam Webster, n.d.). There are two different types of inflammation measured in this study: serum and vascular endothelial measures.
    a. Serum inflammation was measured herein by high sensitivity C-reactive protein (hs-CRP). The hs-CRP is an inflammatory marker associated with adverse cardiovascular events. The hs-CRP measure was derived from the primary dataset and was measured by enzyme linked immunosorbent assay recorded in mg/L as performed by Ortho-Clinical Diagnostics**,** a Clinical Laboratory Improvement Amendment certified laboratory.
    b. Vascular measures of inflammation assess endothelium-dependent vasodilation and were measured in this study by: (a) brachial artery flow mediated dilation (BAFMD); (b) peripheral arterial tonometry derived reactive hyperemia index (RHI), and (c) augmentation index (AI).

i. The BAFMD is a technique that has been widely used to provoke the release of nitric oxide resulting in vasodilation. The BAFMD is quantified as an index of the percentage change in brachial artery diameter from baseline after a blood pressure cuff was inflated for five minutes at 70 mm Hg beyond the SBP of the right arm and was then released to measure a brief high-flow rate known as reactive hyperemia within the brachial artery (Lee et al., 2012; Corretti et al., 2002). In the primary dataset BAFMD reflects the change in brachial artery diameter post-hyperemia, relative to a baseline time which is calculated as a percent wherein 100 x (diameter[peak] – diameter[baseline])/diameter [baseline]) and was recorded in the primary dataset as a continuous number representing this percentage change in diameter (Lee et al., 2012).

ii. The second endothelial measure of inflammation is digital peripheral arterial tonometry (PAT) which is used to derive a reactive hyperemia index (RHI). Peripheral arterial tonometry is an emerging tool that quantifies the reactive hyperemia induced changes in peripheral pulse amplitude tonometry by use of fingertip plethysmographic probes. In this study, all references to RHI refer to a PAT-derived RHI. This RHI measure is indicative of microvascular function and is defined as the dilatory change in a vessel after a blood pressure cuff is inflated around the right forearm for five minutes at a pressure ≥ 70 mm Hg beyond the subject's SBP. This PAT-RHI **(**hereafter RHI) is expressed as a ratio or index comparing baseline to post cuff release of the digital pulse amplitude tonometry signal. In the primary dataset the RHI of the suprasystolic pulse volume amplitude relative to baseline pulse

amplitude was automatically calculated by the EndoPAT software, and was recorded as a continuous variable representing the change in the number of units.

iii. The third measure of inflammation is the augmentation index (AI) which is a measure of arterial stiffness. Augmentation index is a surrogate marker for cardiovascular risk that increases with age and is provided as a percent calculated by the augmentation pressure divided by the pulse pressure x 100% (Fantin, Mattocks, Bulpitt, Banya, & Rajkumar, 2007). In younger subjects, AI is associated with cholesterol level and hs-CRP which implies AI is also a measure of inflammation (Writing Group for the American Heart Association, 2013). Additionally, AI is also associated with SBP and heart rate making it closely correlated with cardiac function and HTN (Nurnberger et al., 2002). In the primary dataset, AI was calculated automatically by the EndoPAT, and was recorded as a continuous variable.

10. Coronary artery disease (CAD) is a symptomatic or asymptomatic disease characterized by atherosclerosis in the epicardial coronary artery(ies) which narrows the vessel lumen and impedes blood flow to the heart (Rimmerman, 2013). In the primary data set, both healthy volunteers and persons with CAD were utilized. In this analysis, patients had either prior CAD, or new onset CAD. New onset CAD was assessed angiographically and was confirmed as CAD when ≥ 50% stenosis existed in at least one major epicardial coronary artery in The University of North Carolina, Chapel Hill Cardiac Catheterization Laboratory (Theken et al., 2012). The primary dataset, however, classified persons as healthy or with CAD (Lee et al., 2012).

11. Depression is a chronic and recurrent mood disorder wherein a person has a depressed mood and/or a loss of pleasure in everyday activities for at least a two week period (Ebert, Nurcombe, Loosen, & Leckman, 2008). In depression, four or more of the following symptoms are present: sleep disturbance, change in appetite or weight, decreased energy or psychomotor activity, decreased concentration, difficulty with decisions, worthlessness or guilt, and suicidal ideation. Depression comes in several diagnostic subcategories which include: major depression, dysthymia, bipolar disorder, cyclothymic disorder, and depression not otherwise characterized (Fauci et al., 2008). In the primary data set, depression was confirmed by a medical diagnosis noted within the medical record.

Specific Aims and Research Questions

The specific aims and research questions to be addressed will include the following:

1. Describe the established stable CAD population's Vitamin D levels, HTN measures and measures of inflammation in those who are and are not depressed.
    a. 1-RQ1: What proportion of the sample is depressed?
    b. 1-RQ2: What are the mean Vitamin D levels, serum hs-CRP level, BAFMD, RHI, and AI in those who are and are not depressed?
    c. 1-RQ3: What proportion of the sample has systolic or diastolic HTN?
    d. 1-RQ4: Is there a difference in Vitamin D levels, SBP, DBP, HTN, BAFMD, RHI, and AI in those who are and are not depressed?
2. Explain the relationship of Vitamin D level to depression
    a. 2-RQ1: Is Vitamin D level associated with depression?
    b. 2-RQ2: When controlling for age, sex, race and BMI, do Vitamin D levels differ in those with and without depression?

3. Examine the relationship of Vitamin D levels, HTN measures (SBP, DBP, yes/no HTN), and serum and endothelial measures of inflammation in those who are and are not depressed.

   a. What is the relationship of Vitamin D levels to measures of HTN (SBP, DBP, yes/no HTN)?

   b. What is the relationship of Vitamin D levels to serum and endothelial measures of inflammation (hs-CRP, BAFMD, RHI, AI)?

   c. Are measures of HTN (SBP, DBP, yes/no HTN) and serum and endothelial measures of inflammation (hs-CRP, BAFMD, RHI, and AI) associated with the occurrence of depression?

   d. When controlling for age, sex, race and BMI, are Vitamin D levels, serum and endothelial measures of inflammation, and measures of HTN associated with depression?

   e. When controlling for age, sex race, BMI and Vitamin D levels, are serum and endothelial measures of inflammation and measures of HTN associated with depression?

Assumptions

This research study was conducted with several assumptions. Research assumptions were: (a) the recording of diagnoses in the medical record (HTN and depression) was complete and correct, (b) those depressed or with HTN had current ongoing treatment, (c) the use of an antidepressant was accurately reflected in the medical record and documented by the investigators, and (d) the equipment utilized provided accurate physiologic measures. Adequate equipment calibrations were assumed to have been performed by Labcorp and Ortho-Clinical Diagnostics to assure that the serum Vitamin D and hs-CRP levels reflected reality, and that the

recommendations of Itamar Medical (Franklin, MA) for calibration of the EndoPAT were sufficient to allow the machine to accurately record data over a three-year period. It was also assumed that the researcher in the primary study used a well-calibrated sphygmomanometer with an appropriately sized blood pressure cuff for each subject. Finally, the research presumes that all physiologic measures were carefully performed by standardized laboratory procedures which adhered to a strict protocol, and the results of all measures were keyed correctly into the data tables.

Advantages and disadvantages exist for any secondary analysis. The main advantage was that existing data can be used to answer new questions. Other advantages include a savings in time and costs, as well as the data collection process was performed with expertise unavailable to the student researcher. However, a major disadvantage is that the data were not collected to answer these specific aims and research questions, so information is not available that might be useful, such as: (a) completion of a standardized depression tool to detect those with newer onset and undiagnosed depression (Boslaugh, 2007), (b) how often the participants are outdoors, (c) if participants used sunscreen daily or irregularly, and (d) if participants took vitamin supplements containing Vitamin D?

Summary

This research study used a secondary analysis of data from adults with CAD to examine the association of their demographic factors, serum Vitamin D levels, measures of blood pressure, and serum and endothelial measures of inflammation with depression. New data were obtained by an analysis of previously frozen serum to ascertain serum Vitamin D levels and liver function. A literature-derived conceptual framework was utilized to guide the study; this framework denotes both direct and indirect associations of serum Vitamin D levels to depression. This study

fills an important public health need by exploring the direct and indirect pathways by which serum Vitamin D levels might influence depression.

CHAPTER II

LITERATURE REVIEW

Introduction

This literature review focuses upon Vitamin D levels and the variables of hypertension (HTN) and serum and endothelial measures of inflammation that are theorized to be associated with depression. There is a proposed direct and indirect relationship between Vitamin D levels and depression depicted in the physiologic model within Figure 1. Specifically, to be discussed are: (a) depression's prevalence and costs, (b) theoretical views of depression, (c) the association of depression with coronary outcomes, (d) the effect of depression treatment upon coronary artery disease (CAD), € the effect of inflammation and HTN upon depression, (f) the effect of endothelial function upon depression, (g) the burden, prevalence and costs of hypertension (HTN), (h) the prevalence of low serum Vitamin D and the effect of demographic factors upon serum Vitamin D level and, (i) the indirect pathways of HTN and serum and endothelial measures of inflammation that may contribute to depression.

Prevalence of Depression in the United States

Depression is a frequently occurring chronic illness that in any one year affects between 6.7% (National Institutes of Mental Health, n.d.) and 9.5% (Centers for Disease Control and Prevention [CDC], 2011c; CDC, 2011d) of the adult population in the United States. Depression is also the leading cause of disability worldwide (World Health Organization, 2012). Lifetime prevalence of mood disorders is high in the United States; 21% will have a mood disorder, about 30% an anxiety disorder, and nearly 17% of adults report a prior episode of major depressive disorder (Kessler et al., 2005).

A study of depression by race and ethnicity within the third National Health and Nutrition Examination Survey (NHANES, 1988-1994) revealed that women have a much higher risk of depression (Riolo, Nguyen, Greden, & King, 2005). Poverty increases the risk of depression, as does race with the prevalence of depression from highest to lowest present in Whites, followed by Mexican Americans, and then, Blacks (National Institutes of Mental Health, n.d.; Riolo, Nguyen, Greden, & King, 2005). The high prevalence of depression between the ages of 30-60 may be partially accounted for by the association of depression with separation, divorce or being widowed (Kessler, et al., 2005). Whites are also high risk because they have an earlier onset of major depressive disorder compared with African Americans (Riolo, et al., 2005). Age of onset typically occurs at 25-26 years for mood disorders, and the lifetime prevalence is lower in those over age 60 ($p < 0.05$) which raises the question of what has changed in society and the environment that fewer older adults report depression (Kessler, et al., 2005). Within the first National Comorbidity Survey in early 1992, 58% of women and 46% of men reported a lifetime occurrence of having a two-week period with a depressed mood (the diagnostic criteria for major depressive disorder). Lifetime prevalence of depression by race was 10.4% for Whites, 8.0% for Mexican Americans, and 7.5% for Blacks (Riolo, et al., 2005) with Kessler et al. (2005) reporting lifetime prevalence of major depression disorder being 16.6%. These statistics show depression as a highly prevalent chronic condition that starts in early adulthood, affects individuals the most between the ages of 30-60 and involves all races, but Whites most heavily.

Theoretical Views on Depression's Origins

Depression is believed to occur due to multiple mechanisms. Altered biology resulting from a vitamin or neurotransmitter deficiency can cause depression (Robinson, 2009). A genetic link exists for depression, which results in those with a family history and certain environmental factors being more likely to become depressed (Breen et al., 2011). Further, there are four other

diseases which are genetically associated with depression: attention deficit hyperactivity disorder, schizophrenia, bipolar disorder and autism (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013). Females have an increased risk of depression because they are 70% more likely to have a lifetime prevalence of depression (National Institutes of Mental Health, n.d.). Other risks for depression include stressful events, grief and loss, some medications, chronic pain, post-partum, and medical conditions such as thyroid disease cancer and coronary artery disease (CAD).

Brain structure and function may also contribute to depression with those having depression noted to have a smaller prefrontal cortex which results in fewer neurons and a lesser ability to manage emotion and executive functions (Fields, 2012). Additionally, there are five genes that have been found to have a lessened expression within the pre-frontal cortex of individuals who become depressed (Fields, 2012). It appears that certain personality types, including those who are aggressive, hostile and less dependent upon others for support may be more at risk for depression and at relapse of depression (Rabasca, 2000). This finding about personality types and depression is supported by studies reporting that a lack of social support is a key factor in predicting depression and relapse (Leahy-Warren, McCarthy, & Corcoran, 2011). In fact, a few decades ago the Type "A" personality, a competitive, aggressive and self-sufficient personality, was linked to CAD (Pozuelo, et al., 2009) which is a condition associated with an increased risk of depression. Indeed, the prevalence of depression is 15-20% in various cardiovascular conditions (Pozuelo, et al., 2009) and occurs in 17-44% of persons with CAD (Khawaja, et al., 2009). These figures in cardiovascular populations reflect substantial variance from the rate of depression in adults within the general population: 6.7% during any 12-month timeframe (National Institutes of Mental Health, n.d.).

Several theories have been discussed in the literature to explain the possible association of inflammation and depression. These hypotheses of depression's origins include: (a) the monamine hypothesis which explains that a deficiency in a balance of neurotransmitters (serotonin and dopamine) may initiate depression (Makhija & Karunakaran, 2013), (b) abnormal negative feedback within the hypothalamic pituitary adrenal (HPA) axis which allows for the increased cortisol found in about 50% of depressed patients which may contribute to depression (Makhija & Karunakaran, 2013), (c) plasticity of the blood-brain barrier in various parts of the brain leads to increased cytokines which may also contribute to depression (Myers, 2008), (d) a complicated cascade wherein damaged neuronal tissues (from perhaps infection or trauma) respond to increased tumor necrosis factor and cytokine release which crosses into the brain via areas devoid of the blood-brain barrier resulting in vagus nerve stimulation and high sympathetic activity which synergistically result in inflammatory effects and possibly altered behavioral responses to illness or injury (Tracey, 2002), and € perturbations of the nitric oxide pathway due to HPA axis abnormalities, increased cytokine activity or activation of the sympathoadrenal system may also contribute toward the development of depression (Rajagopalan et al., 2001).

Additionally, three meta-analyses have found depression to be associated with increased inflammation, but some have pointed out several critiques to this (Dowalti et al., 2010; Howren, et al., 2009; Pasco et al., 2010). Such critiques include that studies have reported: (a) an increase in inflammation associated with depression without consideration of subject's age, sex or body mass index (BMI) all affecting inflammation, (b) the failure of mean cytokine values in multiple studies to be two to three times values found in healthy (non-depressed) controls, and, (c) values for inflammatory markers in the depressed and non-depressed tend to overlap with sometimes the highest inflammatory values found in one of the non-depressed subjects (Raison & Miller, 2011). Yet, there may still be a large physiologic effect from small increases in inflammatory markers,

and some have proposed it may be more "logical" to consider if there may be a sub-type of depression associated with immune dysfunction and inflammation (Raison & Miller, 2011).

Mood has been found to be worse in those with prior cardiovascular events (Peters, et al., 2010). Depressed mood may influence cardiovascular disease leading to associated mortality because of behavior change; the depressed person may exercise less or engage in less preventive care activities (Poole, Dickens, & Steptoe, 2011). Additionally, there may be negative effects from taking antidepressant drugs, such as increased platelet activation or increased blood pressure (Rajagopalan, 2001; Scalco, 2005). Other pathways explaining how depression may lead to poor cardiovascular outcomes include endothelial injury, atherosclerosis or thrombus formation in those not adhering to lifestyle or suggested therapies (Peters, et al., 2010). Other theories linking the proposed association of depression with CAD include: (a) an alteration in the hypothalamic-pituitary axis, (b) overstimulation of the sympathetic nervous system resulting in neuroendocrine dysfunction, and (c) excessive cytokine activity (Poole, et al., 2011; Rajagopalan, et al., 2001). Endothelial dysfunction, which is an inflammatory event, is important to prevent because it is also associated with CAD and may be associated with depression, at least in type 2 diabetics (Wagner, Tennen, Mansoor, & Abbott, 2009). Thus, inflammation is a damaging precursor capable of initiating thrombus formation and HTN which constitute two risks for neuronal injury, which may contribute toward the development of depression.

Sickness Behavior and Vital Exhaustion

Sickness behavior or syndrome is a separate, but overlapping, entity from major depression disorder. Sickness behavior consists of various behavioral responses that allow an individual to adapt to malaise by behaviors such as seeking warmth and conserving energy in response to the illness (Myers, 2008). Likely, there is bidirectional communication between the brain and the immune system which leads to sickness behavior (Myers, 2008; Tracey, 2002).

Sickness behavior is also seen in those on cytokine therapy (such as those being treated with alpha-interferon for hepatitis) and involves depressive symptoms, but is not a psychiatric major depressive disorder (Makhija & Karunakaran, 2013; Raison & Miller, 2011). Cytokines which are associated with sickness behavior increase serotonin reuptake at neural synapses which can lead to depression (Makhija & Karunakaran, 2013). Although this study is not designed to tease out the differences between depression and sickness behavior or their prevalences, the author acknowledges this might be an area for future research with inflammation and Vitamin D as potential predictor variables.

Vital exhaustion (VE) is a construct that originated from work with cardiovascular patients and has been deemed to be an independent risk factor for CAD (Appels, 2004). The domain of depression may overlap that of VE, creating a complicated association (McGowan et al., 2004). Vital exhaustion is a measure of mental health reflective of the states of depression and stress (Hoekstra, Barbosa-Leiker, & Twisk, 2013). Vital exhaustion consists of excessive fatigue, malaise, decreased libido, increased irritability, hopelessness and demoralization (McGowan, et al., 2004; Wojciechowski, Strik, Falger, Lousberg, & Honig, 2000). The Beck Depression Inventory (BDI) which is frequently utilized in studies of persons with cardiovascular disease (Writing Committee for the ENRICHD Investigators, 2003) assesses subjects for many qualities found within those afflicted by VE. The BDI assesses for feelings of guilt, loss of pleasure, tiredness, loss of interest in sex, self-criticalness and worthlessness. Thus, both constructs, VE and depression involve somatic (i.e., fatigue and malaise) and cognitive (i.e., hopelessness) domains (Titov et al., 2011). Stress management programs can decrease VE which co-occurs with periods of prolonged emotional stress or work (Hoekstra, 2013).

Vital exhaustion is important because studies of persons with CAD and myocardial infarction reveal that these persons suffer from fatigue or VE around the time of or after their

cardiovascular event (McGowan, et al., 2004; Wojciechowski, et al., 2000). This clustering of depression and fatigue that occurs around the time of myocardial infarction has been labeled VE (Hoekstra, et al., 2013). Although early research on post-myocardial infarction patients revealed that VE and depression are separate entities (Wojciechowski, et al., 2000), VE and depression were highly correlated in one study ($r = 0.61$, $p < 0.01$) ((McGowan, et al., 2004).

The condition of VE is also important as one considers the model guiding this research (Figure 1) because of the overlap of symptoms between VE and depression. Both VE and burnout predict cardiovascular disease in a large body of research, as well as decreased fertility and poorly rated health, and burnout has been found in women to be positively associated with markers of inflammation that include increased fibrinogen and hs-CRP (Toker, Shirom, Shapira, Berliner, & Melamed, 2005). Additionally, VE has been correlated with increased interleukin-6 (IL-6) which is a measure of inflammation (Janszky, Lekander, Blom, Georgiades, & Ahnve, 2005). A more recent study found comparable VE trajectories in men and women, and within this study levels of IL-6, IL-8 and TNF-α did not vary by type of vital exhaustion (stable preclinical, chronic, or never vitally exhausted) which leaves the association of VE and inflammation less than clear (Hoekstra, et al., 2013). Hence, because VE and depression have been closely correlated, and VE has been correlated at least once with measures of inflammation, it appears reasonable to propose that inflammation may also be associated with depression as the conceptual framework in Figure 1 suggests. In fact, depression and inflammation are likely related because inflammatory biomarkers, including inflammatory cytokines, have been shown to interact with pathophysiologic mechanisms that result in depression (Miller, Maletic, & Raison, 2009).

Costs and Health Outcomes Associated with Depression

Depression affects the lives of individuals and their families in various ways. Depression is associated with increased morbidity and poorer health outcomes (Michelson, 2009) likely because of its association with noncompliance to suggested medical treatment (Black, et al., 2003; Coventry & Gellatly, 2008; DiMatteo, et al., 2000; Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Investigators, et al., 2003; Michelson, 2009; Wing, et al., 2002; Writing Committee for the ENRICHD Investigators, 2003). Depression is also associated with decreased productivity (Khawaja, et al., 2009; Rutledge et al., 2009; Stewart, et al., 2003) and decreased quality of life (Bansil, et al., 2010; Ndokera & MacArthur, 2010). Quality of life can be further affected by treatment non-adherence that is characteristic of those with depression. In fact, persons who are depressed with cardiac disease are less likely to follow through with diet, lifestyle, exercise, testing and follow-up recommendations (American College of Cardiology Foundation, et al., 2012; DiMatteo, et al., 2000; Pozuelo, et al., 2009). Of greater concern, 64% of those with newly diagnosed depression do not fill anti-depressants in 90 days which likely further enhances poorer outcomes (Cantrell, et al., 2011). Thus, there is the possibility that this lack of adherence to depression treatment may also contribute to further treatment non-adherence for other conditions, as well as continued decreased productivity and quality of life for those affected.

In response to the poorer health outcomes found in those with chronic diseases complicated by depression, the United States Preventive Services Task Force in 2009 recommended screening adults for depression when supports are in place to assure an accurate diagnosis, effective treatment and follow-up care (United States Preventive Services Task Force, 2009). Yet, despite a likely increase in depression screening and diagnosis, those who are medically ill, even when their chronic disorders are controlled, continue to report increased

medical symptoms (Pozuelo, et al., 2009). For example, depression enhances the severity of CAD, diabetes and stroke (CDC, 2011b). In those with CAD, depression is also associated with increased risk of heart failure (May, Horne, Carlquist, Sheng, Joy, & Catinella, 2009). Consequently, depression acts as a powerful independent risk factor that worsens health outcomes in chronic disease, especially in CAD.

Beyond the enhanced morbidity and mortality found in those depressed, health care costs are higher in the depressed. Thirty percent of persons one year after myocardial infarctions are depressed as defined by a Beck Depression Inventory score of ≥ 10 (Frasure-Smith et al., 2000; Rutledge, et al., 2009). In one year, costs were nearly doubled in those with depression ($22,960 versus $11,956), and treatment dramatically attenuates health care expenditures from $22,960 to $14,365 (Unützer et al., 2009). Costs for depressed men and women from Canada were 41% higher for depressed versus non-depressed persons one year post myocardial infarction (Frasure-Smith, et al., 2000). In yet another study, treatment diminished women's medical costs post myocardial infarction (Rutledge, et al., 2009). In these women, depression increased costs over five years by 15-53%, and costs varied by use of antidepressants, history of reported treatment for depression and for those ≥ 10 (representing depression) and < 10 on the Beck Depression Inventory II (Beck 2) (Rutledge, et al., 2009). Consequently, with the use of such a low score defining depression, the results of Rutledge et al. (2009) are more surprising because post myocardial infarction costs were higher even in those with a very mild depression (a Beck 2 score of 10). Despite the high costs related to depression and the association of depression with poorer health outcomes, it is unknown at present what physiological factors mitigate the development of depression in CAD patients. Thus, a study of HTN, serum and endothelial measures of inflammation and Vitamin D levels provides insight into an important gap in the literature and may help to improve future outcomes in persons with CAD. New knowledge may allow

clinicians to treat their CAD patients who have HTN or inflammatory markers more aggressively if they deem these individuals to be at a higher risk for depression and the resulting poorer cardiovascular outcomes.

The Association of Depression and Cardiovascular Outcomes

Since 1998, depression has been found to hold at least some level of independent risk for the development of CAD (Mendes de Leon, et al., 1998; Pozuelo, et al., 2009) and in the last years has been firmly believed to worsen cardiovascular outcomes (Goldston & Baillie, 2008). Initially, it was thought that depressive symptoms might not be independent risk factors for heart disease, but that only elderly women in good health might be more at risk when depressed (Mendes de Leon, et al., 1998). Yet, we now know that symptoms of depression are associated with an 81% increase in the incidence of fatal and non-fatal cardiac events in healthy individuals (American College of Cardiology Foundation, et al., 2012), and increased deaths occurred within 6 months in depressed versus non-depressed persons after myocardial infarction (Pozuelo, et al., 2009). In fact, three large, well-designed multi-center trials found that treatment aimed at diminishing the symptoms of depression was not associated with either improved cardiovascular measures or event free survival (Glassman, et al., 2002; Writing Committee for the ENRICHD Investigators, 2003). The first of these trials examined the effect of Sertraline on cardiovascular physiologic measures, which were not improved, even though the drug was deemed safe and effective for recurrent depression in patients with unstable angina or recent myocardial infarction (Glassman et al., 2002). The second study of 2,481 persons with prior myocardial infarction provided an intervention of cognitive behavioral therapy, with group therapy and Sertraline for some, finding that depression and social isolation improved, but there were no significant differences among the treatments in event-free survival (Writing Committee for the ENRICHD Investigators, 2003). In the third large randomized controlled trial (RCT) of depression

treatment of persons with cardiovascular disease, Citalopram was superior over Citalopram plus psychotherapy in reducing depressive symptoms, and the higher the subject's Beck Depression Inventory score, the higher was their five year death rate (Lesperance et al., 2007). Thus, studies illustrate that depression remains a devastating disorder with worrisome effects upon health because even when treated, persons with CAD and depression show no improvement in their cardiovascular outcomes, and there are higher mortality rates in depressed persons with CAD (Khawaja, et al., 2009; Thombs et al., 2008). These landmark trials make it clear that relieving symptoms of depression is not consistently helpful to the heart or survival after the onset of CAD which makes finding and controlling the contributing factors of depression so important.

Depression is a known independent risk factor for CAD (Pozuelo, et al., 2009). Depression not only increases cardiovascular risk, but exacerbates risk factors for CAD such as smoking, diabetes and obesity (Kornerup, Zwisler, Prescott, & Group, 2011). Additionally, medical illnesses predispose individuals to depression, and those with depression report a higher number of medical symptoms (Pozuelo et al., 2009). Consequently, it is possible that numerous medical symptoms may obscure subtle cardiovascular symptoms that a person experiences**,** leading to a lack of recognition of cardiac symptoms and their significance. Another risk for worsening CAD outcomes in those with depression is that persons with depression have platelet activation which enhances thrombosis, as well as impaired brachial artery flow mediated dilation (Pozuelo, et al., 2009; Sherwood, Hinderliter, Watkins, Waugh, & Blumenthal, 2005). Consequently, because of selective serotonin reuptake inhibitors' (SSRI) effects on increasing platelet reactivity (Saran, et al., 2012), and the aforementioned up-regulation of platelet activation and impaired endothelial function in the depressed, it may be very important to prevent depression from ever developing. Once depression develops, it may herald a firestorm of worsening inflammation and cardiovascular risk factors that are difficult to extinguish once

begun. This firestorm of inflammation may be self-perpetuating and reinforcing to the depression, along with the inflammatory reinforcement that may occur due to treatment with SSRIs.

Depression is widely prevalent, costly, damages family health, and leads to poorer health outcomes. Hence, it is important to examine novel factors contributing to this chronic disorder. Depression coupled with CAD increases the risk of fatal and non-fatal cardiovascular events by 81% (American College of Cardiology Foundation, et al., 2012). Only two trials have assessed treatment of depression on cardiovascular outcomes with a long enough follow-up period to usefully assess cardiovascular outcomes: no effect on CV outcomes was found by the treatment of depression (Thombs, et al., 2008; van Melle et al., 2007; Writing Committee for the ENRICHD Investigators, 2003). Trials to date have not shown that treatment of depression in persons with CAD is effective in reducing the cardiovascular endpoints of death and hospitalization after myocardial infarction and sudden cardiac death in those treated (Khawaja et al., 2009). In fact, there may be a lack of a treatment effect due to the low effect sizes in the efficacy trials performed to date (Khawaja et al.,2009). While no harm to cardiac function has been found in those taking selective SSRIs (Thombs et al., 2008), fears remain of antidepressant induced cardiotoxicity (Khawaja, et al., 2009). Consequently, understanding novel risk factors that contribute to depression such as Vitamin D, HTN and/or inflammation may help in the early identification of those likely at risk for depression which may improve outcomes because once depression sets in, outcomes may be forever worsened.

Effectiveness and Outcomes of Depression Treatment

There is controversy regarding the efficacy of antidepressants and psychological counseling (Moncrief, 2002; Murdock, 2012). In a study to determine whether counseling or antidepressants for primary care patients with depression worked better, both treatments were

found to be equally efficacious at eight weeks (Bedi et al., 2000). Part of the debate about what works best for treating depression arose due to methodological problems within studies, as well as a valid concern about a possible amplified placebo effect within mood studies (Moncrief, 2002). At the 10 week follow-up, depression had resolved for the majority (69%) of persons with depression who were aged 18-70 and mostly female (Bedi, et al., 2000). More research has refuted the previously held notion that placebos are as effective as anti-depressants; in fact, 75% of participants with depression respond to anti-depressants (Murdock, 2012).

To date, there remains a lack of clarity as to whether the treatment of depression is effective for persons with CAD (Khawaja, et al., 2009). It is clear that further research is needed on depression and the mechanisms associated with depression. A possible explanation for why those with CAD whose depression is treated do not have better cardiovascular endpoints is that when depression exists, the mood disorder might mask the symptoms of both overt and subclinical CAD, as well as the ability to report or recognize symptoms because the person on antidepressants may have an elevated mood, be more relaxed, or less attuned to subtle bodily cues than persons not taking these agents. Additionally, some anti-depressants could possibly interfere with blood pressure control (such as Venlafaxine) or induce orthostatic hypotension (Amitriptyline) which might partially explain the worsening outcomes in persons who have CAD and concurrent depression (Scalco, et al., 2005). Additionally, SSRI anti-depressants have been associated with disturbed platelet function which creates a pro-thrombotic state (Saran, et al., 2012). Avoiding depression at all costs is of paramount importance since even when treated with SSRIs or tricyclic antidepressants, survival is not improved, and if treatment is resistant to these two classes of drugs, there may be an even higher risk for CAD morbidity and mortality (Saran, et al., 2012).

Inflammation

Inflammation is a nonspecific, compensatory bodily response to cellular injury. Inflammation is characterized as a protective physiological reaction that involves "capillary dilatation, leukocyte infiltration, redness, heat, pain, swelling," and often loss of function that serves as a mechanism initiating the elimination of noxious agents and of damaged tissue (Merriam Webster, n.d.). Mediators of inflammation cause one component event to occur through a receptor, causing the initiation of an inflammatory cascade (Chauhan, 2006). There are many factors which mediate inflammation, including interleukins, platelet activating factor, eicosanoids, tumor necrosis factors, complement proteins, bradykinin and others (Chauhan, 2006). Serum measures of inflammation include high sensitivity C-reactive protein (hs-CRP), erythrocyte sedimentation rate, fibrinogen, homocysteine, CD-40 ligand, complement, uric acid and inflammatory cytokines such as interleukin-6 (IL-6), interleukin-1 (IL-1), and tumor necrosis factor-alpha. Within the serum, there are measures of acute and chronic inflammation. One measure of acute inflammation is hs-CRP which is a globulin that is an abnormal protein detectable during acute inflammation. The hs-CRP is believed to be important in assisting complement to mark damaged cells so that phagocytosis ensues.

Associations between Measures of Inflammation and Depression

There is mounting interest in a hypothesis that inflammation contributes to the pathogenesis of depression (G. E. Miller & Cole, 2012). Depression is known to involve inflammatory cytokines, such as IL-6 (Harrison et al., 2009). In fact, an inflammatory pathway, and perhaps one specifically triggered by hs-CRP, may be the link between depression and CAD because the steps of atherosclerosis are known to involve inflammation (Kop, et al., 2002; Zahn et al., 2013). It is concerning that treatment of depression with SSRIs (Escitalopram and Fluoxetine) in those recently diagnosed with the mood disorder has been found to be effective in

lowering inflammatory markers, including hs-CRP, independently of their treatment effect (Chavda & Kantharia, 2010).

High Sensitivity C-reactive Protein. Elevated hs-CRP is an acute phase protein associated with higher risk for CAD, which is a condition associated with HTN (Howren, et al., 2009; Strandberg & Tilvis, 2000). Studies have elucidated with consistency that high levels of hs-CRP are associated with elevated risk for major coronary events in men, women, and the elderly (Pearson, et al., 2003). Over 50 prospective studies worldwide have established the association of hs-CRP with both fatal and non-fatal coronary events, including strong associations with stroke, CAD and vascular mortality (Boekholdt & Kastelein, 2010). This may be due to the increase in waist circumference which is associated with inflammation because adipose tissue is metabolically active and may mediate the secretion of cytokines (Ackermann et al., 2011; Hoekstra, et al., 2013). Non-physically fit individuals who are at risk for CAD often have increased waist circumference which has been correlated with increased markers of inflammation such as hs-CRP, fibrinogen and erythrocyte sedimentation rate (all p values < 0.01) (Rogowski et al., 2010).

Brain inflammation occurs as a result of altered perfusion within a setting of abundant influencing factors that include environment, genetics, trauma, excessive stress, as well as metabolic and autoimmune disorders which can enhance the progression of inflammation. Depending upon the neuronal effects of these multiple and unique influencing factors, there is a loss of homeostasis which can take the form of multiple mood disorders (Saavedra, 2012). Both HTN and atherosclerosis lead to a narrowing of the arterial lumen and decreasing blood flow to body tissues which results in further inflammation (Gonzalez & Selwyn, 2003; Thuillez & Richard, 2005). Consequently, HTN, atherosclerosis, and inflammation can contribute to neuronal damage which may affect mood (Cheung et al., 2005; Cha, Patel, Hains & Mahan,

2011). So, an association between hs-CRP and depression is possible because brain inflammation is capable of disrupting auto-regulatory mechanisms and altering cerebral blood flow which can result in edema and/or excessive sympathetic tone which leads to vasoconstriction and decreased cerebrovascular tone (Saavedra, 2012).

Increased hs-CRP has been linked to depression by numerous studies (Howren, et al., 2009; Kop, et al., 2002; Ma, et al., 2011). A convincing association between hs-CRP and depression in persons over 65 years was established by Kop et al. (2002), in middle aged healthy volunteers by Ma et al. (2011), and in adolescents transitioning towards depression (G. E. Miller & Cole, 2012). Conversely, a study of persons with chronic obstructive pulmonary disease did not show an association between hs-CRP and depression, but did show a weak positive correlation between depression and another measure of inflammation, tumor necrosis factor alpha (TNF-α) (Al-shair et al., 2011). In contrast to these findings, the level of hs-CRP did not differ by sex in a recent sample that was mainly middle aged, White and educated; however, hs-CRP did correlate with depression in individuals who had no life-threatening illnesses (Ma, et al., 2011). For example, Ma et al. (2011) found that individuals with higher depression scores also had higher hs-CRP levels. In a United States sample (N = 4,268) of persons of a mean age 72 who were 84% White and 15% African American, depression was associated with elevated hs-CRP (3.51 versus 3.31; unadjusted p = 0.0008, adjusted p = 0.056; (Kop, et al., 2002).

A recent study examined the association of depression with hs-CRP in four groups: diabetics with and without depression, depressed individuals without diabetes, and healthy controls (Zahn et al., 2013). The investigators explored the relationships between hs-CRP and atherogenic measures of platelet inflammation (CD40) with a surprising finding that hs-CRP was not associated with depression (Zahn, et al., 2013). Admittedly, this was a small trial of less than 100 subjects which may not have been representative of the larger population; the study utilized a

cross-sectional design, and there may have been a ceiling effect in the diabetic patients with depression affecting results because hs-CRP is known to be higher in diabetics. As a result, not all studies have consistently shown a relationship between hs-CRP and depression.

Inflammation may also be highly prevalent in persons with CAD. In a recent systematic review of trials conducted between 1967 and 2008 to establish the relationship between hs-CRP, IL-6, IL-1 and depression, the authors found hs-CRP, IL-1 and IL-6 to vary positively with depression in clinical and community samples (Howren, et al., 2009). For the studies of depressed persons with CAD (9 trials), the effect size of hs-CRP was low (d = 0.18) but significant (p = 0.02; (Howren, et al., 2009). The authors also found that there was no significant gender difference between depression, exhaustion (not VE) nor hs-CRP, although hs-CRP was elevated in those with exhaustion (Howren, et al., 2009).

In summary, it has been hypothesized that inflammation contributes to depression and that the strong linkage between depressed persons with CAD and elevated hs-CRP may be due to the influence of inflammation stimulating atherogenesis. It appears reasonable to examine the association of various measures of inflammation, including hs-CRP, within a sample of persons with CAD to explore the relationship between inflammation and depression.

Endothelial Measures of Inflammation. The endothelium is a single-cell layer of continuous cells that line blood vessels separating the blood from the vessel wall; it is affected by chemical toxins such as cigarette smoke, air pollution, and disease states including septic shock, increases in blood pressure, hyperlipidemia and diabetes. The endothelium secretes chemicals (nitric oxide, prostacyclin and endothelin) that regulate blood pressure, coagulation and platelet activity (Tousoulis, et al., 2005). Nitric oxide is a potent vasodilator. When the endothelium malfunctions, it does not secrete appropriate amounts of nitric oxide to meet physiologic requirements for blood pressure control. Thus, endothelial dysfunction is an indirect measure of

inflammation because it heralds reduced vasodilation, as well as a concurrent pro-inflammatory state (Endemann & Schiffrin, 2004). Thus, dysfunction of the endothelium is associated with HTN, a hallmark of CAD and cardiovascular diseases, (Endemann & Schiffrin, 2004), and endothelial dysfunction is proportionally related to an increased incidence of cardiovascular events (Tousoulis et al., 2005). Not surprisingly, reduced endothelium-dependent vasodilation is also present in atherosclerosis (Gonzalez & Selwyn, 2003) which is another hallmark finding in persons with CAD. There is a reduced cerebral perfusion in those with HTN and atherosclerosis due to decreased cerebral blood flow that can cause neuronal injury and result in mood disorders, including depression (Saavedra, 2012). Thus, not surprisingly, evidence suggests that endothelial dysfunction is a marker of inflammation that occurs in depression, and for this reason, measures of endothelial dysfunction are important when examining inflammation's indirect contribution toward depression (Do, Dowd, Ranjit, House, & Kaplan, 2010; Rajagopalan, et al., 2001).

Brachial Artery Flow Mediated Dilation and PAT as Measures of Inflammation. Relatively new technology has made the measurement of endothelial dysfunction possible (Corretti et al., 2002; Flammer et al., 2012). A brachial artery flow mediated dilation (BAFMD) has been widely used to provoke the release of nitric oxide resulting in vasodilation which is then measured after a cuff is released that was inflated for between 5 and 10 minutes at approximately 50 mmHg above the subject's baseline SBP. The BAFMD is quantified as an index of the percentage change in brachial artery diameter from baseline to various times after the release of the occluding blood pressure cuff to measure a brief high-flow rate known as reactive hyperemia within the brachial artery (Corretti et al., 2002; Lee, et al., 2012). Brachial artery flow-mediated dilation provides a measure of endothelial function and is important because it is associated with the severity and progression of CAD, as well as CAD risks (King, 2004).

Peripheral arterial tonometry (PAT) is a non-invasive measurement of endothelial dysfunction performed by the use of a fingertip probe which measures pulse amplitude which is reflective of the health of the microvasculature. During the measurement of PAT, often a reactive hyperemia index (RHI) or RHI-PAT (herein by an EndoPAT machine and called RHI) is calculated and this RHI-PAT reflects the ratio of the digital pulse volume during reactive hyperemia divided by that measured at baseline (Rubinshtein et al., 2010). A low RHI index detected by PAT, such as that provided by an EndoPAT device (Itamar Medical) is consistent with endothelial dysfunction and CAD (Rubinshtein et al., 2010).

Digital PAT measures may approximate the basal blood flow within the brachial artery more so than a reactive hyperemia index, with one study revealing no relation between RHI-PAT ratio and BAFMD (Lee, et al., 2012). This result of no correlation between PAT and BAFMD makes it less than clear what place BAFMD and PAT individually have in predicting clinical outcomes (Lee, et al., 2012), and if these measures will be correlated only in certain populations (Kuvin et al., 2003; Rubinshtein, et al., 2010). For example, Rubinshtein et al. (2010) utilized a large outpatient sample of mean age 54 that was half women, half of whom smoked and more than that had hyperlipidemia, and found that BAFMD and PAT were correlated. Kuvin, Mammen, Mooney, Alsheikh and Karas (2007), in a mostly male outpatient sample of mean age 54, half of whom had CAD, found that in those with two or more cardiac risk factors, both BAFMD and RHI-PAT responses were significantly lower, and that those with CAD had both lower BAFMD and PAT responses than those without CAD (Kuvin, A., Mooney, Alsheikh-Ali, & Karas, 2007). In contrast, Lee et al. (2012) found no correlation between RHI-PAT and BAFMD in subjects with either CAD or healthy volunteers although the CAD group was older (59 years versus 51 years) than the healthy volunteers and also heavier. Having a heavier cohort of CAD patients may be an important factor to study because older age is already known to be

associated with more inflammation (Ershler & Keller, 2000; Krabbe, Pedersen, & Bruunsgaard, 2004). Additionally, increased BMI in many cases is associated with a higher waist circumference which is also associated with more inflammation and endothelial dysfunction (Ackermann, et al., 2011; Lilitkarntakul et al., 2012). Persons with CAD are heavier which makes the finding of no correlation between BAFMD and RHI-PAT somewhat surprising, but this may have occurred due to the careful attention in this study to measurement precision by Lee et al. (2012). Thus, it appears early in our understanding of these relatively new endothelial measures, and BAFMD and RHI-PAT results may vary by populations studied.

Augmentation Index. Augmentation index (AI) is a reflection of the augmentation pressure which is obtained from an arterial wave form propagating forward during systole and coming back from the periphery which reflects elasticity or arterial stiffness (Fantin, et al., 2007). Augmentation index is defined as the ratio of central augmented pressure to the central pulse pressure, and it can be measured in several arteries in various manners (Shimizu & Kario, 2008). Women have a higher AI, and AI is higher centrally, in the carotid versus radial arteries (Fantin, et al., 2007). Other factors which influence AI include age, heart rate, height (Janner, Godtfredsen, Ladelund, Vestbo, & Prescott, 2012) and diastolic blood pressure (DBP) (Fantin, et al., 2007).

Central blood pressure measurements are thought to be good predictors of cardiovascular disease (Janner, et al., 2012; Shimizu & Kario, 2008) because the AI increases significantly in the presence of increased cardiovascular risks (Nurnberger, et al., 2002). Also, large artery stiffness correlates with time to myocardial ischemia (Kingwell, Waddell, Medley, Cameron, & Dart, 2002). For example, another measure of arterial stiffness other than AI, pulse wave velocity, has been found to vary inversely with the hyperemic response measured by BAFMD (Laurent et al., 2006; Malik, Kondragunta, & Kullo, 2008). More important evidence is accumulating that AI is

associated with current depression or anxiety which may enhance the progression of atherosclerosis and cardiac disease, although the relation of AI to anxiety appears to be stronger than to depression (Seldenrijk et al., 2013). Consequently, increasing AI may be a novel risk factor for cardiovascular disease; however, some current evidence questions its clinical utility (Kario & Shimizu, 2008). It may be useful to ascertain if AI does correlate in this CAD sample with depression, or with BAFMD which has been shown to be decreased in those with depression (Sherwood, 2005). One additional factor shedding other doubts on AI is that it is unclear how EndoPAT calculates the AI and if the AI has utility in predicting cardiovascular risks or events according to C. Lee (personal communication, July 15, 2013).

Peripheral Arterial Tonometry and Endothelial Dysfunction

Peripheral arterial tonometry (PAT) is a non-invasive endothelial measure that is capable of measuring reactive hyperemic changes within blood vessels. It is possible that depression is an important contributor to endothelial dysfunction specifically in CAD, or that perhaps some abnormality found in persons with CAD (atherosclerosis or HTN which are both inflammatory events) may be contributing to depression. Digital PAT measures approximate the *basal* blood flow within the brachial artery more so than a reactive hyperemia index, and the parent study from which this analysis is drawn revealed no relationship between RHI-PAT ratio and BAFMD (Lee, et al., 2012). Thus, future studies will be needed to compare the findings of PAT and BAFMD within the same populations to determine if the measures are reliably correlated and if they definitively predict coronary or depression risk.

Endothelial Measures of Inflammation and Depression

In a May, 2013 literature search within EBSCO's Academic Search Complete using a Boolean search strategy, only 15 articles were found when searching concurrently the terms depression, endothelial and flow-mediated dilation. Within this literature, negative mood

(described as greater anxiety in women) was associated with a lesser flow mediated dilation (Schott, Kamarck, Matthews, Brockwell, & Sutton-Tyrrell, 2009). The mood state of healthy adults is associated with BAFMD (Denise C. Cooper et al., 2010), and middle and older aged persons with CAD who had Beck Depression Inventory Scores at the accepted cut-score of 10 or more for depression (Chen et al., 2009) were also found to have attenuated BAFMD (Sherwood, et al., 2005). Of interest in this later study, treatment of these subjects with CAD with the use of antidepressant medications (mostly SSSRIs) was associated with an improvement in the BAFMD scores ($p < 0.05$) (Sherwood, et al., 2005). This latter finding is important to note given that SSRIs do not attenuate worsened coronary outcomes, possibly due to the platelet dysfunction associated with their use.

In a meta-analysis of 12 studies ($N = 1,491$) designed to determine the association of depressed mood with flow-mediated dilation (unclear if all studies used solely BAFMD), diverse populations, including persons with CAD, were determined to have an inverse correlation between depressed mood and endothelial function (D. C. Cooper et al., 2011). Additionally, in a study of postmenopausal women with type two diabetes and recurrent depressive disorders, history of depression (even when in remission) was associated with endothelial dysfunction (Wagner, et al., 2009). There is an inverse correlation between negative mood and BAFMD. Antidepressant medication improves BAFMD, possibly lessening inflammation which is a contributing factor to depression (D. C. Cooper, et al., 2010; Dantzer, O'Connor, & Freund, 2008; Schott, et al., 2009; Sherwood, et al., 2005).

The vascular endothelium plays a central role in the body in various inflammatory processes that are related to CAD (Tousoulis, et al., 2005). Brachial artery flow-mediated dilation (BAFMD) is an important measure of endothelial function because of its known association with CAD risks and the severity and progression of CAD (King, 2004). The

connection between depression and CAD has been hypothesized to exist due to abnormalities of either the hypothalamic pituitary axis, the sympathetic nervous system, or cytokine activation because all three of these systems prevent the endothelium from secreting nitric oxide (Rajagopalan, et al., 2001). The first study to demonstrate abnormal endothelial function in young, healthy depressed patients compared with matched controls found that reactive hyperemia results were attenuated in depressed persons compared to the control group (Rajagopalan, et al., 2001). However, the response to a dose of nitroglycerin was comparable in both groups despite chemokines (including E-Selectin and Monocyte Chemoattractant Protein-1) being significantly different in those with and without depression (Rajagopalan, et al., 2001). This study pointed out that Paroxetine, a SSRI, worsens endothelial function by increased platelet aggregation (Rajagopalan, et al., 2001). The finding of altered platelet function in those taking selective serotonin reuptake inhibitors (SSRIs) may be the reason that even when depression is present, identified and treated, improvement in depression has not been shown to improve cardiovascular outcomes (American College of Cardiology Foundation, et al., 2012; Glassman, et al., 2002; Writing Committee for the ENRICHD Investigators, 2003). Consequently, depression (whether treated or not) likely exists due to and within an inflammatory state which helps to explain the poor outcomes typically seen in CAD patients who are depressed (Pozuelo, et al., 2009; Saran, et al., 2012).

It appears that those who are healthy, have CAD, or have diabetes all show an attenuated BAFMD response when depressed–even a remote history of depression results in attenuation of the BAFMD (Wagner, et al., 2009). Sherwood et al. (2005) found that use of antidepressant medication (75% of his sample) was associated with improved BAFMD, yet others have found that patients receiving treatment of depression did not ameliorate the altered endothelial dysfunction when measured by BAFMD (Rajagopalan et al., 2001). Accordingly, there are

complex pathways at play within the endothelium and neurons that are at present not fully understood. As a result, it appears appropriate to assess multiple endothelial measures in a study evaluating the prevalence of depression in order to confirm that BAFMD and PAT are correlated as has been previously suggested, as well as to assess their association with depression (Kuvin, et al., 2003; Rubinshtein, et al., 2010).

Hypertension

This model analyzes blood pressures and the prevalence of a HTN diagnosis in persons with CAD. The conceptualization in this review of literature is that blood pressure measures and hypertension are synonymous.

Hypertension is a public health concern because of its growing prevalence in the United States. Between 1999 and 2009, the death rate due to HTN increased by 17.1% nationally (American Heart Association Statistics Committee and Stroke Statistics Committee et al., 2012). Epidemiologic data support an increased risk of stroke, renal disease and cardiovascular disease when systolic and diastolic blood pressures increase above normal parameters (Hajjar, Kotchen, & Kotchen, 2006). Prevalence of HTN in adults within the United States increases with age with 38% of those 45-54 having HTN, 52% at ages 55-64, and after age 65 present in 71% of women and 64% of men (Writing Group for the American Heart Association, 2013). More disturbing is that another 6%-7% of adults have undiagnosed HTN which translates to an overall prevalence of approximately 4 of 10 adults in the United States being afflicted by HTN (Writing Group for the American Heart Association, 2013). This high prevalence of HTN in the United States is alarming because only 78% of persons diagnosed with HTN are taking medication for their HTN, and merely 65% have their HTN controlled (Writing Group for the American Heart Association, 2013). When a first myocardial infarction or stroke occurs, those individuals have a respective 69% and 77% prevalence of HTN (Heart Disease and Stroke, 2013). Thus, a large number of

undiagnosed and sub-optimally managed persons with HTN are at risk for CAD and health events or conditions associated with HTN and CAD.

Hypertension is also a complex disease associated with poorer health outcomes (Writing Group for the American Heart Association, 2013). Multiple contributors have been associated with HTN, albeit inconsistently, including CAD, depression and even Vitamin D deficiency. Vitamin D likely affects HTN because Vitamin D receptors (VDRs) are present within vascular smooth muscle and cardiomyocytes (Bhandari et al., 2011). Inflammation has been proposed as the bridge linking HTN and atherosclerosis (J. J. Li & Chen, 2005). This is likely because of the increased activity of the renin-angiotensin system (RAS) which places oxidative stress upon the heart and vasculature and up-regulates inflammatory mediators in a setting of increased blood pressure leading to endothelial dysfunction (J. J. Li & Chen, 2005). Hypertension may be an independent risk factor for depression indirectly, because HTN leads to both endothelial dysfunction and an inflammatory state, both of which result in neuronal injury and subsequent mood disorders (Meng, et al., 2012). As a result, the pathogenesis of HTN should be fully understood to decrease the severe impact of this highly prevalent condition and its associated 48equel, including depression.

Outcomes and Costs Associated with Hypertension

A shorter life expectancy is found in those with HTN, and outcomes for those with HTN are notably worse, especially in the setting of poor control. This is likely because of the confirmed increase in CAD, stroke and mortality associated with SBP over 120 mm Hg (Hajjar, Kotchen, & Kotchen, 2006). Coronary disease is reduced 21%, cardiovascular mortality 25% and overall mortality 13% in those whose SBP is reduced 12-13 mm Hg over four years (Cushman, 2003). Thus, reducing blood pressure has profound effects, as further evidenced by the suboptimal treatment of SBP being associated with increased hospitalizations and complications

from associated conditions (Esposti & Valpiani, 2004). Of note, there is an established link between non-compliance and increased healthcare expenditures of those who have HTN, and of increased complications such as heart failure, cerebrovascular disease and CAD (Esposti & Valpiani, 2004). For example, of those with a first myocardial infarction or stroke, 69% and 77% respectively have HTN (Heart Disease and Stroke, 2013). This non-compliance in those with HTN was evaluated in a study of California Medicaid claims; 86% of newly initiated HTN drug regimens were interrupted in the first year (Esposti & Valpiani, 2004). This non-compliance with treatment of HTN resulted in an increased cost of $873 per patient year, but the figure was $1,840 for patients who had been previously hospitalized (Esposti & Valpiani, 2004). So, HTN remains a costly public health threat because of its wide prevalence, poor outcomes, and the excessive dollars spent to treat the conditions and the conditions associated with it.

Hypertension, Coronary Artery Disease and Inflammation

Subsequent to the occurrence of HTN, left ventricular hypertrophy, atherosclerosis, CAD and eventual heart failure occur. Persons with heart failure and CAD have the highest percentage of emergency room visits (Cantrell, et al., 2011). Per year total costs (medical plus pharmacy) for HTN patients are $476, but this figure balloons to $3,307 for persons with heart failure and $3,789 for persons with CAD (Cantrell, et al., 2011). Consequently, because CAD results in more emergency room visits and hospitalizations than HTN, managing HTN well improves coronary health outcomes and makes good fiscal sense (Cantrell, et al., 2011).

Vitamin D

Vitamin D is a fat soluble vitamin with numerous physiologic effects throughout the body due to the plethora of VDRs present within multiple tissues which include, among many, the vascular endothelium, cardiomyocytes and the brain (A. Zitterman, 2006). Some have called Vitamin D a neurosteroid (Mellon, Griffin, & Compagnone, 2001) or a neuroactive steroid

(Kiraly, Kiraly, Hawe, & Makhani, 2006) because the brain is considered a steroid producing organ similar to the testes and adrenal glands. Another steroid within the brain is angiotensin II (Ang II) which can be produced there. Additionally, the brain's VDRs allow for abundant enzyme activity for metabolizing pre-Vitamin D into calcitriol (Bertrone-Johnson, 2009). Thus, steroids synthesized within the brain, such as Ang II and Vitamin D, are considered neurosteroids.

The central renin-angiotensin system (RAS) was discovered in the 1990s when it was discovered that most organs were capable of locally synthesizing Ang II (Saavedra & Benicky, 2007). Angiotensin II affects blood pressure because its secretion leads to vasoconstriction and increased blood pressure, and when a state of low serum Vitamin D is present, Ang II is increased (Y. C. Li, et al., 2004). Additionally, the neurotransmitter, dopamine, is important for the regulation of mood, and calcitriol (active Vitamin D) and may alter neurotransmitter (dopamine) synthesis and storage to affect mood (Eyles, Burne, & McGrath, 2012). Thus, because the brain is an important binding site for Vitamin D and rich in neurotransmitters that assist in the regulation of mood, it is not surprising that low serum Vitamin D has been associated with depression and severe mental illness (Ganji, et al., 2010; Gracious, Finucane, Friedman-Campbell, Messing, & Parkhurst, 2012; Hoogendijk et al., 2008; May, et al., 2010).

Deficiencies of Vitamin D early in life may result in schizophrenia and other disorders (Barnard & Colón-Emeric, 2010; Gracious, et al., 2012; Kesby, Eyles, Burne, & McGrath, 2011), and Vitamin D remains important throughout the body because of the vitamin's numerous extra-skeletal effects (Barnard & Colón-Emeric, 2010). For instance, long term deficiencies are associated with a number of adverse outcomes including increases in depression, Parkinson's disease, Alzheimer's disease and cognitive decline (Kesby, et al., 2011). Vitamin D blocks angiotensin receptors decreasing stress, anxiety and brain inflammation (Saavedra, et al., 2011).

The blockage of angiotensin-1 receptors (AT_1) is considered neuroprotective and assists in decreasing Alzheimer's and improving cognition. The rationale for the neuroprotective role of Vitamin D is that improved cerebral perfusion occurs when Vitamin D status is adequate and the renin-angiotensin system (RAS) is not overly stimulated (Saavedra, 2012; Saavedra, et al., 2011). Thus, Vitamin D likely has effects upon brain development, maintenance of cognition and neuronal function, mood, and possibly depression (Eyles, et al., 2012; Kesby, et al., 2011).

Organ Function Affecting Vitamin D

Vitamin D is initially an inactive pro-hormone that undergoes processing within the liver followed by the kidney to become metabolically active Vitamin D (calcitriol) (Ross, Taylor, Yaktine, & Del Valle, 2011b). Vitamin D enters the circulation after conversion of 7-dehydroxycholesterol in response to ultraviolet light contacting the skin; it is converted in the liver to 25-OHD (pre-Vitamin D) which is the serum level typically measured for this proposed study and for clinical screening. This 25-OHD is next converted in the kidney into active Vitamin D, calcitriol, when there is a lack of calcium in the body. Hence, because the liver and kidney are involved in the body's conversion of an inactive pro-hormone into a metabolically active Vitamin D, it appears plausible and reasonable that adequate liver and kidney function are needed if one expects the body to have the capability to produce sufficient Vitamin D. In fact, there is an association between 25-OHD and liver dysfunction, with low 25-OHD levels predicting hepatic decompensation (Putz-Bankuti et al., 2012). Additionally, Vitamin D deficiency is found universally in those with chronic kidney disease (Bansal, Bansal, Mithal, Kher, & Marwaha, 2012), and renal function is also imperative in order to synthesize active Vitamin D efficiently (Cronin, 2010).

Prevalence of Vitamin D Deficiency in the United States

A recent secondary analysis of NHANES surveys remarked upon a decrease in Vitamin D deficiency levels between the 1988-1994 and the 2001-2004 NHANEs surveys (Ginde, Liu, & Camargo, 2009). However, these findings should not be taken as reflecting a definitive trend because of the reported problems with NHANES Vitamin D analyses making comparison of survey years inaccurate due to known assay drifts during the time these Vitamin D serum analyses (25-OH(D) were run (Centers for Disease Control and Prevention, 2010). Despite this lack of clarity in the reliability and validity of NHANES Vitamin D results, it is known that Vitamin D deficiency is highly prevalent with 1 billion people worldwide estimated to have vitamin D deficiency or insufficiency (Holick, 2007; Holick, et al., 2011; Ross, et al., 2011). Ginde, Liu and Camargo (2009) reported that 55% of those in the 1988-1994 NHANES had insufficient or deficient Vitamin D (< 30 ng/ml). Forrest and Stuhldreher's examination (2011) of the most recent NHANES that has available Vitamin D levels (2005-2006) revealed that 82% of Blacks and 69% of Hispanics were deficient when defined as Vitamin D ≤ 20 ng/mL. Another analysis of 2005-2006 NHANES data reported that Vitamin D ≤ 30 ng/mL was present in 97% of Blacks and 91% of Mexican Americans; this number varies from that of Forrest and Stuhldreher (2011) because a broader definition of Vitamin D deficiency was utilized (< 30 ng/mL); this latter study only calculated a level for Mexican Americans because there were so few other Hispanics with Vitamin D levels in the 2005-2006 NHANES sample (Puglisi & McCoy, 2013).

Demographic Factors that Influence Vitamin D Levels

Known risks for Vitamin D deficiency include older age, non-White race, obesity, those with limited sun exposure, those with malabsorptive diseases or bariatric surgery, frequent use of sunscreen or occlusive clothing (Barnard & Colón-Emeric, 2010), and those living north of 37th parallel, which runs along the border of North Carolina and Virginia (Forrest & Stuhldreher,

2011; Harvard Medical School, 2008; Holick, 2007). Demographic factors such as increasing age, minority status and increased BMI are all known to influence serum Vitamin D levels by lowering them. Minority race and Latino ethnicity usually are associated with enhanced dermal melanin which competes for ultraviolet light and reduces the conversion of 7-dehyroxycholesterol into precursor Vitamin D, thus lowering serum Vitamin D levels (Holick, 1995). There is an inconsistent relationship of Vitamin D and sex (Holick, 2007; Ross, et al., 2011); studies to date do not show a strong predilection for either sex being associated with increased vitamin D insufficiency (Elamin, et al., 2011).

Obesity affects serum Vitamin D levels by sequestration of Vitamin D within body fat which also lowers serum Vitamin D levels (Holick, 2007; Holick, et al., 2011), and studies have found significant differences in the serum Vitamin D levels of normal weight, overweight and obese subjects (Forrest & Stuhldreher, 2011). In general, studies have shown those with a higher body mass index (BMI) have lower serum Vitamin D levels and may require higher daily supplementation doses once their Vitamin D is corrected because of the extra adipose tissue sequestering Vitamin D (Holick, et al., 2011). An indirect measure of obesity is BMI. Body mass index is a fairly reliable estimate of body fatness that correlates with underwater weighing. Because BMI misguides the estimation of fatness in athletes, some researchers use underwater weighing to estimate fatness. In underwater weighing, a person with more body fat will be more buoyant than the lean athlete. Consequently, it is possible for lean, muscular athletes who are weighed on land to have a falsely high BMI despite their not having much body fat (CDC, 2011a). An important determinant of those with lower Vitamin D status is increased BMI, and the consistency between Vitamin D being inversely related to BMI holds regardless of-sex or age (Vimaleswaran et al., 2013).

Direct Effects of Vitamin D Level upon Depression

Vitamin D receptors (VDRs) are found in multiple areas of the human brain which is likely the reason that Vitamin D has been explored as a possible preventative or therapeutic for depression. Of the sixteen studies reviewed examining the association of Vitamin D and depression, 5 cross-sectional studies, a secondary analysis of the third National Health and Nutrition Examination Survey (Ganji, et al., 2010), one prospective cohort study (Tolppanen et al., 2012), a retrospective chart review (May, et al., 2010), and two randomized double blinded placebo controlled trials of Vitamin D (Jorde, et al., 2008; Lansdowne & Provost, 1998) support an inverse association between serum Vitamin D level and depression (Hoogendijk, et al., 2008; Knippenberg, Bol, Damoiseaux, Hupperts, & Smolders, 2011; Milaneschi et al., 2013; Stewart & Hirani, 2010; Wilkins, Sheline, Roe, Birge, & C., 2006).

Vitamin D deficiency affects both children and adults. Fourteen of the aforementioned studies involve adults, but Tolppanen et al. (2012) studied children with a mean age of 10 and found that low Vitamin D early in childhood appeared to predict later depression. Two new studies of Vitamin D in children give credence to the argument that Vitamin D deficiency may be related to mood. In the first, adolescents presenting for acute mental health treatment had their 25(OH)D level examined, and it was found that adolescents presenting with psychotic features had significantly lower Vitamin D levels ($p = 0.04$; (Gracious, et al., 2012). Those adolescents who had deficient Vitamin D were more likely to be Asian or Black, and Vitamin D deficiency increased from December through March although no seasonal effects were identified ($p = 0.14$; (Gracious, et al., 2012). The other study involving children and Vitamin D established that an association between Vitamin D and depression begins in childhood that is independent of other confounders, with higher Vitamin D_3 at age 9.8 years associated with a lower relative risk of symptoms of depression at age 13.8 years (Tolppanen, et al., 2012). Of interest, serum Vitamin

D_2 concentrations were not associated with depressive symptoms in this British study; this study reflects the tendency in European countries to measure Vitamin D_2 and D_3 separately, so one must interpret such studies with some caution, since in the United States, a Vitamin D level consists of total Vitamin D_2 and D_3 (Tolppanen et al., 2012).

Four of the 16 aforementioned studies revealed no significant association between vitamin D level and mood, of which two were randomized controlled trials (Dean et al., 2011; Kjaergaard et al., 2012), one a cross-sectional study from the 2005-2006 National Health and Nutrition Examination Survey (Zhao, Ford, Li, & Balluz, 2010) and the fourth study explored mental well-being and Vitamin D levels (Sanders et al., 2011). This fourth trial was a double blinded RCT which evaluated an intervention of Vitamin D_3 and mental well-being showing no significant effect of an annual 500,000 IU fall dose of Vitamin D_3 in women over the age of 70 (Sanders, et al., 2011). A criticism of this study is that the tools utilized to measure well-being (the General Health Questionnaire-12, the World Health Organization Well-being Index and the Patient Global Impression Improvement tools) likely represent a much broader array of one's health status than would be measured on most depression tools.

The smaller RCT showing no association between Vitamin D levels and depression involved 128 patients aged 18-30 in Australia who were supplemented daily for six weeks with Vitamin D^3 5000 IU; the study revealed no beneficial effect on depression scores as measured by the Beck Depression Inventory (Beck) (Dean, et al., 2011). However, there were only ten participants of 128 in this study with low Vitamin D levels which means if these subjects were spread amongst the experimental and control groups, this would likely result in less than the statistically desirable 80% power. Additionally, the study ran for only 6 weeks which is approximately one half of the three months needed for Vitamin D levels to reach steady state

(Dean, et al., 2011). It is questionable if adequate time elapsed within this 6-week study to hold Vitamin D levels steady at a therapeutic level to affect Beck depression scores.

The larger RCT reflecting no association between serum Vitamin D levels and depression involved a nested case-control study and an interventional study (n = 243) which utilized placebo or 40,000 IU of Vitamin D_3 given weekly for 6 months (Kjaergaard, et al., 2012). The nested case-control study of those with Vitamin D levels below 55 nmol/l and above 70 nmol/l involved adults aged 30-75 years, concluding that there was an association between low levels of Vitamin D and depressive symptoms, but that there was no effect of supplementation (Kjaergaard et al., 2012). Again, given the half-life of Vitamin D is around 20 days, this causes steady state to be reached at about 90-100 days, so a strength of this 6 month study is that an additional 3 months passed when the intervention group should have had maximum therapeutic effect, and yet, no effect upon depression scores was found.

There are, however, two positive RCTs involving supplementation with Vitamin D_3. In the larger second RCT, there was an association between Vitamin D levels and depression in adult subjects who received either doses of 20,000 IU, 40,000 IU or placebo weekly for a year (Jorde, et al., 2008). Depression was again assessed by the Beck but this time, a statistically significant association was found between total Beck scores ($p < 0.01$) and scores for items 1-13 and 14-21 ($p < 0.05$). Findings of this year-long study were weakened by 25% attrition which may have affected the study's power and subsequent findings, and may also have been weakened because the laboratory analysis only measured serum 25(OH) D_3, and not D_2 and D_3 as is the custom in the United States. Of course, the clinical significance of a drop in a Beck score of 1 to 2 points is questionable, yet the study was strengthened by its yearlong intervention which allowed the participants to reach and then remain at steady state Vitamin D level which may have influenced the findings.

Lansdowne and Provost (1998) performed a very early RCT that revealed an inverse association of serum Vitamin D level and depression. This study utilized 44 healthy college students who were given either placebo, 400 IU or 800 IU of Vitamin D_3 for only *five days* in the late winter. The finding was that the positive affect (measured by The Positive and Negative Affect Schedule, 1988) was enhanced in both intervention groups beyond the control group (Lansdowne & Provost, 1998). The negative effect of this scale reportedly measures depression, state anxiety and general distress. Of interest even though the half-life of Vitamin D_3 is 20 days, comments were made by subjects taking Vitamin D_3 for only five days that they felt "really good" whereas no such comments were made by the control group (Lansdowne & Provost, 1998). A criticism of this study is that the authors did not disclose if any blinding procedures were utilized, so this lack of control may have contributed to the glowing comments made by the experimental groups at such an early time frame (Lansdowne & Provost, 1998). Additionally, a retrospective chart review of 59 patients who had participated in other studies on fatigue and depression found that Vitamin D status negatively correlated with depression (r = - 0.326, p = 0.006), although this effect was weakened in a regression that controlled for age, disability status and fatigue scores (Knippenberg, et al., 2011).

Three secondary analyses have been performed on large national samples within the United States and England, with two of the three (Ganji, et al., 2010; Stewart & Hirani, 2010) finding an inverse relationship between Vitamin D level sufficiency and depressive symptoms (Zhao, 2010). The third study included subjects from the 2005-2006 National Health and Nutrition Examination survey (n = 3,916) and found no significant association between serum Vitamin D levels and the presence of minor, major or moderate-to-severe depression (Zhao, et al., 2010). These three studies utilized different tools to evaluate depression; the two studies finding an inverse association between depression and Vitamin D levels utilized the Geriatric Depression

Scale and the Diagnostic Interview Schedule, while the non-significant study utilized the Patient Health Quesstionnaire-9 depression screening tool (Ganji, et al., 2010; Stewart & Hirani, 2010; Zhao, et al., 2010). The samples were drawn from highly divergent populations with Ganji et al. (2010) utilizing non-institutionalized subjects aged 15-39 within the United States, Stewart and Hirani (2010) utilizing a nationally drawn English sample greater than or equal to 65 years old, and Zhao et al. (2010) utilizing widely contrasted ages of greater than or equal to 20 years. The various ages of the samples and the different tools utilized makes drawing comparisons difficult. Certainly studies finding the association of low Vitamin D levels with depression are hard to interpret since they may suffer reverse causality because depressed individuals are less likely to go outdoors and exercise which would result in endorphin release and also result in exposure to increased sunlight which might enhance dermal production of Vitamin D (Eyles, et al., 2012). Of course, another potential confounder of study results is that decreased Vitamin D levels can cause primary hyperparathyroidism which is known to be associated with mood disorders, including depression, that improves after treatment of the hyperparathyroidism (Hoogendijk, et al., 2008). Further cross-sectional studies would be useful in another sample of widely divergent ages controlling for the aforementioned confounders utilizing a standard tool such as the Beck and another standard tool, such as the PHQ-9. This would help to support the strength and direction of the relationship between serum Vitamin D levels and depression.

Of the remaining studies reviewed, strong support for an association between Vitamin D levels and depression comes from a recent large study of healthy controls, and those with current or remitted depression (n = 2,386). The authors found lower Vitamin D levels in the currently depressed, with the lowest levels in the most severely depressed (p = 0.001, Cohen's d = 0.44; (Milaneschi, et al., 2013). These authors even concluded that correcting low Vitamin D levels might be cost-effectively utilized as a prevention strategy or as a therapy for depression

(Milaneschi, et al., 2013). Another cross-sectional trial of Vitamin D levels and depression in subjects aged 65-95 showed that Vitamin D levels were 14% lower in those with minor depression and major depressive disorder than in unaffected subjects ($p < 0.001$) (Hoogendijk, et al., 2008). Another study of older adults (≥ 60 years) found Vitamin D level was associated with impairment of mood that was measured by the clinician's diagnosis and a depression symptoms inventory, based upon the elements within the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (Wilkins, et al., 2006). Vitamin D levels have been associated with a worse mood and higher depressive symptoms in older adults who have deficient and insufficient Vitamin D levels; with a low Vitamin D level (< 20ng/mL) being associated with an odds ratio (OR) of 11.69 (for the deficient) and 2.54 (for the insufficient) for higher depressive symptoms when age, sex, race and season were controlled (Wilkins, et al., 2006). These three findings in the elderly of an association between low Vitamin D levels and depression (or mood) appear to support the contention that there is a significant association present in older adults (Hoogendijk, et al., 2008; Laurent, et al., 2006; Stewart & Hirani, 2010; Wilkins, et al., 2006).

Thus, most studies support an inverse association between Vitamin D levels and depression in populations of various ages. While studies may have suffered from reverse causality, had methodological flaws, or unequal interventions of Vitamin D supplementation (some with or without calcium or with varying dosages), there is still a stronger body of evidence supporting a direct association between low Vitamin D levels and depression, and a treatment effect of Vitamin D upon depression.

Indirect Pathways of Vitamin D Contributing to Depression

In the proposed model guiding this research (Figure 1), Vitamin D levels have two indirect pathways that contribute to depression. These include the influences of low serum Vitamin D levels upon HTN and inflammation which may contribute to an increased depression

prevalence. These indirect influences upon Vitamin D level by HTN and inflammation will be explored next.

Vitamin D and Hypertension

Cross-sectional studies and a few RCTs are beginning to show an association between Vitamin D levels and HTN. The literature about the association between blood pressure and Vitamin D levels is likely affected and complicated by many factors, including: (a) fluctuations in normal and abnormal Vitamin D laboratory values between the earlier and later NHANEs trials and at various commercial labs (Centers for Disease Control and Prevention, 2010; Pollack, 2009), (b) use of different serum Vitamin D tests (25 OH(D) versus 1,25(OH)D), (c) various supplementation interventions studied (with and without calcium; (Jorde, Sneve, Torjesen, & Figenschau, 2010; Judd, Raiser, Kumari, & Tangpricha, 2010; Pfeifer, Begerow, Minne, Nachtigall, & Hansen, 2001; R. Scragg, Khaw, & Murphy, 1995), (d) various supplementation doses and products within the few randomized controlled trials (Judd, et al., 2010; Pfeifer, et al., 2001; Ross, et al., 2011); and, € studies varying in their reporting of either SBP or DBP versus an endpoint of HTN or normotension (A. K. Gupta, et al., 2011).

Thus, there remains a lack of consistency in the evidence supporting the association between Vitamin D levels and blood pressure. There have been multiple secondary analyses of Vitamin D levels from National Health and Nutrition Examination Survey (NHANES) data (A. Fraser, Williams, & Lawlor, 2010; R. Gupta et al., 2010; R. Scragg, Camargo, & Simpson, 2010). To date, only a few randomized controlled trials of Vitamin D supplementation exist that assess Vitamin D's effect upon blood pressure (Jorde, et al., 2008; 2010; Judd, et al., 2010; Pan, Wang, Li, Kao, & Yeh, 1993; Pfeifer, et al., 2001; R. Scragg, et al., 1995). Several secondary analyses explore the association of serum Vitamin D level with blood pressure, and all found an inverse association between blood pressure and serum Vitamin D levels (A. Fraser, et al., 2010; A. K.

Gupta, et al., 2011; R. Scragg, et al., 2010). However, another study, which was a 7 year longitudinal study of postmenopausal women from the Women's Health Initiative, found no mean difference in SBP and DBP, although notably the supplementation intervention included only Vitamin D 400 IU (cholecalciferol) which is below the current recommended daily allowance of 600 IU for those ages 1-70 and 800 IU for those over age 70 (Margolis et al., 2008; Ross, et al., 2011). What remains in question about Vitamin D levels and blood pressure is: (a) whether elevated SBP or DBP is associated with low Vitamin D levels, (b) for which populations Vitamin D level affects SBP (perhaps only those with really low serum Vitamin D levels or only in Blacks) (Puglisi & McCoy, 2013), (c) if assay drifts in Vitamin D levels during previous studies influenced findings, and (d) if the season of the year the serum Vitamin D level was drawn affects findings because few studies report this important variable, although one found no association of Vitamin D level upon season (Gracious, 2012).

National Health and Nutrition Examination Survey data (NHANES) data from 1988-1994 and 2001-2006 demonstrated that heart rate and SBP are significantly higher in those whose serum Vitamin D levels were insufficient or deficient (defined as <15 ng/mL; (Scragg, et al., 2010). However, results were weakened in this trial since both NHANES III (1988-1994) and the 2000-2006 NHANES trials had serum Vitamin D test assays that drifted numerically because DiaSorin's radioimmunoassay test was reformulated during that time frame (Ross, et al., 2011; Scragg, et al., 2010). Users of NHANES data have been cautioned to not make direct comparisons between NHANES III (1988-1994) data and NHANES 2000-2006 (NHANES, 2010). Another secondary analysis of NHANEs data (N = 3,958) was conducted which found that again Vitamin D levels were inversely associated with SBP ($p < 0.001$), but not significantly with DBP ($p = 0.19$) (A. Fraser, et al., 2010).

A pre-diabetic sample of White subjects with pre-hypertension had lower serum Vitamin D than normotensive subjects (A. K. Gupta, et al., 2011). Another study of Whites found a three-fold higher prevalence of confirmed HTN in a solely male sample with serum Vitamin D levels that were within the current definition for a Vitamin D deficiency state (Burgaz et al., 2011). While this latter study had a strong design utilizing manual and 24 hour ambulatory blood pressure readings, it was weakened by the use of subjects taking anti-hypertensive medications (Burgaz, et al., 2011).

There have been five RCTs on Vitamin D's effect on blood pressure (Jorde, et al., 2008; Judd, et al., 2010; Pan, et al., 1993; Pfeifer, et al., 2001; R. Scragg, et al., 1995), and one five year trial is underway to assess the effect of Vitamin D and omega-fatty acid supplementation on HTN and cancer risk (Manson et al., 2012). Three of the five RCTs evaluated did not find a significant association between Vitamin D supplementation and blood pressure; however, there are weaknesses in each of these trials (Jorde, et al., 2008; Pan, et al., 1993; R. Scragg, et al., 1995). In the most recent RCT of the three, no significant association was found between Vitamin D levels and blood pressure, but a limitation to the study was the huge dropout rate (25%) in subjects who had significantly lower Vitamin D levels ($p < 0.001$) ((Jorde, et al., 2008). The second RCT without significant findings involved an intervention of 100,000 IU given once in December to residents of Great Britain aged 63-76 whose Vitamin D levels were measured five weeks later (R. Scragg, et al., 1995). A limitation of this study is that the five week follow-up may not have allowed enough time for the effects of having a sufficient Vitamin D level to manifest within and remain at a steady state in the vascular smooth muscle (R. Scragg, et al., 1995). Another early supplementation trial that ran for only 11 weeks found no association between Vitamin D, SBP and DBP (Pan et al., 1993). While this was a blinded RCT trial,

subjects received only 200 IU of Vitamin D which is known to be much lower than current recommended daily allowances of 600-800 IU for adults (Pan, et al., 1993; Ross, et al., 2011).

In contrast, two RCTs have found an association between Vitamin D level and blood pressure (Judd, et al., 2010; Pfeifer, et al., 2001). Another study found a significant effect of Vitamin D level and calcium over 15 weeks upon both blood pressure and plasma lipids in middle aged adults (43.6 years); there might have been a robust treatment effect demonstrated had the intervention (200 IU of Vitamin D within Caltrate + D) been more in line with necessary recommended daily allowances for Vitamin D (Major, Alarie, Dore, Phouttama, & Tremblay, 2007). Another RCT was an eight week study of older subjects (mean age 74) that found that Vitamin D with calcium was more effective than the calcium alone in suppressing SBP (Pfeifer, et al., 2001). A later supplementation trial demonstrated an association between Vitamin D levels and blood pressure, showing in the calcitriol (D_2) group a 9% reduction in SBP versus placebo ($p < 0.001$) (Judd, et al., 2010). It is, however, difficult to accept this latter study as robust science because it had merely 9 subjects with two dropping out, and the groups varied at randomization (Judd, et al., 2010). Despite these criticisms of the studies to date, an emerging trend of an association between higher SBP in those with lower serum Vitamin D levels has now been supported in two RCTs (Judd, et al. 2010; Pfeifer et al., 2001) and has also been found observationally in multiple secondary analyses of NHANEs data (A. Fraser, et al., 2010; R. Gupta, et al., 2010; Judd, et al., 2010; Pfeifer, et al., 2001; R. Scragg, et al., 2010).

Further RCTs are needed at the current recommended daily doses per age to assess if Vitamin D is a very inexpensive way to decrease blood pressure, the incidence of HTN and subsequent heart or cerebrovascular disease. While the evidence is early and is still accumulating on Vitamin D's impact on blood pressure, the Endocrine Society in their June, 2011 publication recommended that supplementation with Vitamin D to prevent falls in the elderly is reasonable.

However, neither the Endocrine Society nor others yet recommend supplementation to prevent cardiovascular disease, alter mood or to improve quality of life (Holick, et al., 2011; Wuerzner, Burnier, & Waeber, 2012). Others have recommended Vitamin D supplementation for public health benefits as well as for its anti-hypertensive effects because of the numerous body systems it helps, limited toxicity, and because trials have failed to show significant findings in the anti-hypertensive effects of Vitamin D in normotensive individuals with normal Vitamin D levels making supplementation's benefit not readily apparent (Pilz, Tomaschitz, Ritz, & Pieber, 2009).

The Association of Vitamin D Levels, Hypertension and the Brain

Inadequate Vitamin D status is linked with an up-regulated renin-angiotensin system (RAS) system (Y. C. Li, et al., 2004), and is associated with HTN and risk of HTN due most likely to either excess cortisol or renin secretion (Forman, et al., 2007). The RAS system contributes to HTN because renin secretion leads to aldosterone secretion, salt retention and eventual creation of angiotensin II (Ang II) which exerts effects as a potent vasoconstrictor to raise blood pressure (Y. C. Li, et al., 2004). Evidence of how Ang II can affect the cardiovascular system by increasing the heart-rate and blood pressure is long-standing, but Ang II also has multiple other bodily effects beyond the body's central reaction to stress (Szczepanska-Sadowska, Cudnoch-Jedrzejeska, Ufnal, & Zera, 2010). Angiotensin II is a *brain* regulatory peptide (Saavedra, 2010). The known effects of Ang II include: (a) a direct action on blood vessels which results in vasoconstriction, (b) production and release of anti-diuretic hormone, which increases water re-absorption in the kidney, and (c) stimulation of aldosterone synthesis and excretion, which enhances renal sodium absorption and affects the brain by increasing thirst (Martini & Wood, 2008). A classic and detailed review of the brain RAS is within Phillips and de Oliveira (2008), but in brief, Ang II binds to Angiotensin I (AT_1) causing vasoconstriction and results in nitric oxide release. Next, Ang II receptor stimulation leads to vasodilation, and

Angiotensin 4 (AT_4) receptor stimulation affects learning and memory. Therefore, Vitamin D can block stimulation of angiotensin receptors, and it is the stimulation of AT_1 receptors that contributes to HTN (Copstead & Banasik, 2010; Szczepanska-Sadowska et al., 2010).

The RAS is activated by low serum Vitamin D levels. The earliest evidence that the RAS system might affect mood came from studies involving the effects of Captopril (Ciobica, Bild, Hritcu, & Haulica, 2009). Captopril increased mood which suggested that the *brain* RAS might interfere with acetylcholine release and production of Ang II disturbing cognitive function; therefore, drugs blocking the RAS are likely to be anxiolytic and/or antidepressant (Ciobica, et al., 2009; Saavedra, 2012). It is now believed that the effects of angiotensin converting enzyme inhibitors (ACE-i) and Ang II antagonists provide more than blood pressure control, but have significant effects upon cognition and mood (Liu et al., 2012; Saavedra, 2010; Saavedra, et al., 2011). This is likely because cardiovascular disease affects not only the heart and peripheral organs, but also the brain which has its own RAS (Saavedra, 2012). Consequently, drugs blocking the RAS system (ACE-inhibitors and ARBs) are anxiolytic and anti-depressant in nature and may also improve quality of life (Saavedra, 2012; Saavedra, et al., 2011). The human brain is influenced by the effects of aging, genetics and the environment. When brain inflammation occurs, neuronal injury occurs, which can lead to depression, cognitive loss and impaired neurological performance (Saavedra, 2010, 2012). Reducing RAS stimulation within the brain is important, because decreasing Ang II leads to decreased inflammation and neuronal injury, and an increased amount of substance P. Substance P degrades amyloid plaques, which are characteristic pathological findings in Alzheimer's disease (Saavedra, 2012). The effect of reduced RAS stimulation and increased substance P is accordingly neuroprotective.

Evidence is beginning to explicate Ang II's effects in creating chronic stress and inflammation which can also lead to mood disorders, and in particular, depression (Liu, et al.,

2012; Saavedra, 2012; Saavedra et al., 2005). Multiple brain mechanisms including the classical neurotransmitters (epinephrine, norepinephrine, serotonin, and GABA), and classic neuropeptides such as Ang II and newly discovered neuropeptides (orexins, apelin, leptin, tumor necrosis factor-alpha TNF-α, ghrelin) interact among the neural circuits to regulate the cardiovascular system. However, disturbances in the release or action of these neuropeptides is what gives rise to HTN, chronic stress, depression and other psychiatric disorders (Saavedra, 2010; Szczepanska-Sadowska, et al., 2010). When Ang II is bound within the brain to AT receptors, it leads to increased thirst, an increased salt appetite, sympathetic activation (which can affect mood) and the release of vasopressin (Bader & Ganten, 2008). Thus, Ang II bound to AT_1 receptors in the brain creates a vulnerability toward cerebrovascular ischemia (from decreased blood flow) and brain inflammation, both of which can predispose the injury of neurons (Saavedra, 2010). Consequently, the suppression of AT_1 receptors by Ang II receptor blockers (ARBs), therefore, has a neuroprotective effect (Saavedra, 2012).

In sum, evidence to date has illustrated that there is an increase in the expression *and* activity of Ang II in those who are Vitamin D deficient, and that increased levels of Ang II raises cardiovascular and HTN risks (Y. C. Li, et al., 2004; Szczepanska-Sadowska, et al., 2010). Support for an increased cardiovascular risk due to RAS stimulation and decreased cardiac hypertrophy comes from animal research (Xiang et al., 2004). Studies with mice lacking VDRs show these mice have behavioral impairments; on the other hand, rats with Vitamin D deficiency have a significant decrease of dopamine, a neurotransmitter implicated in depression (Bertrone-Johnson, 2009). The brain and peripheral Ang II systems are stimulated during stress, and blockage of brain AT_1 receptors results in decreased anxiety and stress-induced alterations in behavior (Saavedra, et al., 2005). Thus, stimulation of AT_1 receptors by Ang II causes

vasoconstriction which is a key component to ischemia, inflammation and neuronal injury which could influence mood and may contribute to depression.

Vitamin D Levels' Indirect Contribution to Depression through Hypertension

It has been apparent for decades that hypertensive vascular disease affects not just the heart and kidneys, but also the brain (Hollander, 1976). Consistently elevated SBPs over-ride the auto-regulatory mechanisms within the brain which leads to decreased cerebral blood flow that injures neurons (Cha, et al., 2011; Saavedra, Julius, & Zhou, 2006). Hypertensive effects in the brain are known to cause infarcts, angiopathy and even cerebral atrophy which can affect cognition and mood.

Hypertension is associated with endothelial injury which commences an inflammatory cascade (Endemann & Schiffrin, 2004). In addition, Saavedra, Benicky and Zhou (2006) suggested that protection of the brain from ischemia may not be tied solely to blood pressure, but due to inhibition of the RAS, adding that inhibition of AT_1 receptors is associated with enhanced nitric oxide formation and vasodilation (Saavedra, et al., 2006). If so, enhanced nitric oxide secretion would be a defense mechanism of a working endothelium that is attempting to maintain neuronal homeostasis and function; but, the endothelium would be expected to be damaged from HTN, making it less effective or ineffective in secreting nitric oxide.

The association of Vitamin D and HTN has been discussed, and the strongest evidence appears to support that SBP, which occurs during the force of myocardial contraction perfusing the brain, is likely associated with Vitamin D status (Judd, et al., 2010; Major, et al., 2007; Pfeifer, et al., 2001; Wu, Ho, & Zhong, 2010). Because HTN injures fragile cerebral neurons beyond the altered function already present in depression, having HTN concurrently with depression may augment the neuronal injury and dysfunction. The theoretical model in Figure 1 predicts there is an indirect pathway from which low Vitamin D influences depression indirectly

via a pathway involving HTN. Together, HTN and depression worsen health outcomes because of the associated decreased executive function and treatment adherence in those with both conditions (DiMatteo, et al., 2000; Goldston & Baillie, 2008; Khawaja, et al., 2009). These decreased abilities were possibly at play when a study found that integrating treatment interventions for depression and HTN improved health outcomes; the subjects with integrated treatment had less depressive symptoms and a lower SBP (Bogner & de Vries, 2008).

While HTN may be associated with depression, the literature is unclear about the populations at risk for having both conditions and the cause of the variables' association (Hildrum, et al., 2011; Meng, et al., 2012). Depression and HTN may be associated due to either genetics or environmental stress initiating the conditions (R. Fraser, et al., 1999; Grewen, et al., 2004). One model has proposed that dietary Vitamin D and calcium in the setting of renal failure (which admittedly is often caused by, or at least associated with, HTN) leads to vascular calcification, ischemia, and neuronal cell death (Payne, Anderson, & Steffens, 2008). Yet others have proposed that the physiologic mechanism linking depression to HTN involves abnormal function of the autonomic nervous system, wherein those with depression have increased sympathetic tone and poor vagal control (Scalco, et al., 2005). Certainly, it appears clear that there is a physiologic mechanism at play between HTN affecting neuronal injury leading to depression and perhaps other mood disorders (Saavedra, 2012).

In a study of persons over age 80 with HTN, there was a strong association of depression with all cause and cardiovascular mortality, as well as with stroke and all cardiovascular events (Peters, et al., 2010). The authors commented that depression is common after stroke, heart attack and in early dementia and raised the specter of reverse causation: Does depression precede or follow the stroke, heart attack and dementia (Peters, et al., 2010)? Hence, in contrast to the theoretical model proposed herein where low Vitamin D concurrent with HTN contributes to

depression, Peters et al. (2010) proposed that depression may contribute to HTN. In this conceptualization, depression might influence HTN due to poor lifestyle behaviors such as less exercise, a less stringent diet resulting in the effects of hyperlipidemia. Endothelial injury may be related to depression in some patients, including those with CAD (Do, et al., 2010; Ellis et al., 2012). Other authors have explored this alternate pathway of depression leading to HTN. A recent meta-analysis involved depression and HTN incidence in apparently healthy normotensive persons in prospective cohorts utilizing an end point of the incidence of HTN (Meng, et al., 2012). Nine studies met the inclusion criteria, and the authors found in the 22,000 subjects who were followed for nine years that depression increased the risk of HTN by an adjusted relative risk of 1.42 (95% CI [1.09, 1.86], $p = 0.009$) ((Meng, et al., 2012). This likely occurred because HTN is associated with endothelial injury which commences an inflammatory cascade (Endemann & Schiffrin, 2004).

Inflammation clearly affects the brain. Brain inflammation occurs as a result of altered perfusion within a setting of abundant influencing factors that include environment, genetics, trauma, excessive stress, metabolic and autoimmune disorders which can enhance the progression of inflammation. Thus, atherosclerosis, which is highly associated with HTN, is also an inflammatory event. Like HTN, atherosclerosis leads to a narrowing of the arterial lumen, decreasing blood flow to body tissues, resulting in inflammation (Gonzalez & Selwyn, 2003; Thuillez & Richard, 2005). Consequently, the model in Figure 1 theorizes that blood pressures and serum and endothelial measures of inflammation may be associated with depression, and this effect may be due to neuronal damage (Cha, et al., 2011; Cheung et al., 2005; Ellis, et al., 2012).

The Association of Vitamin D and Inflammation

Vitamin D has a role in supporting immune function, and when impaired, animal models have shown the acquisition of various autoimmune diseases (Manson, et al., 2012). Thus, it

comes as no surprise that Vitamin D deficient persons have an increased risk for autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, diabetes and Crohn's disease (Ardizzone, Cassinotti, Bevilacqua, Clerici, & Porro, 2011; Guillot, Semerano, Saidenberg-Kermanac'h, Falgarone, & Bossier, 2010; Hayes, Cantorna, & DeLuca, 1997; Holick, 2007; Manson, et al., 2012). For instance, there has been an association established between multiple sclerosis (MS) and the latitude of residence of those afflicted. The belief is that risk of having a low serum Vitamin D level increases when the sun's zenith angle is altered (as occurs in northern latitudes), thus, decreasing skin synthesis of Vitamin D. Adequate serum Vitamin D level may lower MS risk, and there is a decreased risk of MS among the offspring of mothers who have high Vitamin D levels (Simon, Munger, & Ascherio, 2012). White women and men who have increased serum Vitamin D levels have a lower MS risk, and Vitamin D protects against the development of MS (Holick, 2007; Simon, Munger, & Ascherio, 2012). Thus, it appears that Vitamin D can impede the development of various inflammatory conditions.

It is unclear if supplementation reverses the association between inflammation and Vitamin D level. Serum and endothelial measures of inflammation were measured in a 12 week RCT involving Vitamin D supplementation of patients with type 2 diabetes. Neither hs-CRP, oxidative stress markers, BAFMD or pulse wave velocity were positively influenced by an intervention of 5,000 IU of Vitamin D_3 daily for 12 weeks (Yiu et al., 2013). Because it takes about 90-100 days until steady state is reached for Vitamin D levels, re-assessing serum levels is usually done after at least 3 months. It is possible a 12 week time frame (such as that employed in Yiu et al., 2013) was not long enough for the Vitamin D level to normalize and to exert its beneficial effects within the tissues, receptors and the immune system.

Several molecular mechanisms have been proposed as to how low Vitamin D levels may be associated with increased inflammation (Guillot, et al., 2010; Querfeld, 2013). Guillot et al.

(2010) proposed that Vitamin D might have immunomodulating effects due to its action upon T-cells and B-cells, and further proposed that Vitamin D might hold promise for future treatments of "dysimmune" diseases. Querfeld (2013) proposed that low Vitamin D level is associated with increased inflammation through several mechanisms:

- Shortened leukocyte telomeres which are associated with low levels of Vitamin D and high sensitivity C-reactive protein (hs-CRP);
- Increased hs-CRP levels, which are independently associated with anemia in kidney disease, which is considered to be a by-product of inflammation;
- Dis-inhibition of TNF-α converting enzyme so that renal cells release more TNF-α and other inflammatory markers into the circulation;
- Increased albuminuria which is inflammatory and a known risk for cardiovascular disease and mortality that occurs when Vitamin D is low; and,
- Repressed nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling through VDRs which results in decreased inflammation via a reduced infiltration of pro-inflammatory T-cells.

It is clear that Vitamin D is involved in physiologic pathways that involve both inflammation and the immune system. A National Institutes of Health funded five-year prospective Vitamin D supplementation trial of 16,000 participants will assess for vascular and cancer endpoints (Manson, et al., 2012). In another study, long-chain n-3 polyunsaturated acids (PUFA), which are found in fish oils and thought to decrease inflammation, were supplemented to assess their effect upon Vitamin D status (Itariu, Zeyda, Leitner, Marculescu, & Stulnig, 2013). This trial involving PUFA supplementation showed that serum Vitamin D level was negatively correlated with hs-CRP and IL-6 at baseline, and that treatment with PUFA did not affect Vitamin

D status. However, supplementation did affect some inflammatory markers, including IL-6 and EPA (eicosapentaenoic acid), and as predicted, the control group retained its inverse association of Vitamin D, IL-6 and hs-CRP throughout the trial (Itariu, et al., 2013). Thus, how Vitamin D level affects inflammation and what measures of inflammation are affected remains unclear.

Vitamin D Levels' Indirect Contribution to Depression through Inflammation

The model in Figure 1 proposes an indirect pathway with Vitamin D level being associated with inflammation and depression. There is some debate if serum markers of inflammation are higher in those who are depressed (Howren, et al., 2009), with some finding an association between serum markers for inflammation (hs-CRP, IL-6, and cellular adhesion molecules) and low Vitamin D (Guillot, et al., 2010; Querfeld, 2013). Elevated hs-CRP may exist 6 months beyond the resolution of depressive symptoms (G. E. Miller & Cole, 2012), but observational studies have been inconsistent about the association of Vitamin D level with serum measures of inflammation (Amer & Qayyum, 2012; Ewers, Gasbjerg, Zerahn, & Marckmann, 2007). It has been postulated that the influence of Vitamin D level upon inflammation may occur due to its modulation of cytokine genes which are controlled by the VDR (Amer & Qayyum, 2012). Adding to the confusion, studies have not shown a consistent response of supplementation to amelioration of the inflammation (Amer & Qayyum, 2012; Milaneschi, et al., 2013).

There is a relationship between active Vitamin D level or calcitriol [1,25(OH)2D_3] and endothelium dependent vasodilation, but it believed that it is the calcitriol that affects the endothelium to release nitric oxide release (Rostand, 2010) likely because Vitamin D is involved in the biosynthesis of nitric oxide synthase. The direction of this relationship is important because the primary study utilized an intervention of a sublingual nitroglycerin tablet in order to assess endothelium-dependent vasodilation. This was done about half way through the vascular

measurement procedures. After the sublingual nitroglycerin, subjects were allowed to lay flat and recover for usually ten minutes thereafter before being walked to the laboratory. There is no way to know how long they waited for their blood draw; the primary study's principle investigator, Dr. Craig Lee, believes there were at least thirty (30) minutes between the nitroglycerin administration and the serum laboratory draw for most subjects (C. Lee, personal communication, August 26, 2013). Thus there was plenty of time for the nitric oxide to disappear within the blood because it's half-life in aqueous solutions is 445 seconds, but this number drops significantly in hemoglobin solutions (Hakim, Sugimori, Camporesi, & Anderson, 1996). Regardless of this, it is the active Vitamin D (calcitriol) that affects the amount of nitric oxide release, not the other way around, and there is no evidence that nitric oxide release degrades serum Vitamin D level.

In a recent study undertaken to establish the role of Vitamin D levels upon inflammation, serum Vitamin D and hs-CRP levels were assessed within a sample of adults from NHANES 2001-2006. Amer and Qayyum (2012) hypothesized that perhaps the beneficial effect of Vitamin D would occur only in those with the lowest Vitamin D levels, and not those with adequate levels. They found an inverse relationship between Vitamin D levels < 21 ng/ml and elevated hs-CRP, but as hypothesized, after a 21 ng/ml Vitamin D level was surpassed, the associated hs-CRP levels increased, suggesting that excess Vitamin D might be pro-inflammatory (Amer & Qayyum, 2012).

In contrast, others found that in persons undergoing renal transplant, an admittedly special population that utilizes anti-rejection drugs, there was no statistically significant correlation between mean serum Vitamin D level, calcitriol, kidney function (by estimated glomerular filtration rate) and hs-CRP despite that the kidney is the site of hydroxylation of Vitamin D into active Vitamin D (calcitriol) (Ewers, et al., 2007). Of course, some persons having renal transplant may have had an inflammatory cause, such as systemic lupus. Despite

this lack of association with hs-CRP in renal patients, a study of women more than 65 years old with hip fractures that had serum measures of Vitamin D and IL-6 assessed found that women deficient in Vitamin D had higher IL-6 levels in the year post fracture (Miller et al., 2007). These IL-6 levels appeared to increase over the baseline, 2, 6 and 12 month assessments (Miller, et al., 2007). The authors concluded that they were uncertain if the Vitamin D deficiency explained the variability in the women's inflammatory state fully, and called for further research of those with Vitamin D deficiency to help understand if the vitamin deficiency explained the women's adverse outcomes (Miller, et al., 2007).

Summary

The brain RAS was endorsed by molecular biology in the 1970s, when angiotensin forming tissues were found in vascular smooth muscle (Bader & Ganten, 2008; Phillips & de Oliveira, 2008). This brain RAS differs from the traditionally understood peripheral RAS system of the kidney. However, because of the blood-brain barrier, most Ang II receptors cannot be reached by circulating peripherally produced Ang II, which means that the Ang II receptors *in the brain* are effectively activated by locally synthesized Ang II which functions as a neuroactive steroid (Bader & Ganten, 2008). There is an established inverse relationship between Ang II and Vitamin D level (Li et al., 2004). The link likely occurs because the VDRs and RAS systems are within the same tissues, and there is most probably a feedback mechanism between the brain's RAS and its VDRs (Ferder, Inserra, Manucha, & Ferder, 2013; Y. C. Li, et al., 2004). Thus, low serum Vitamin D is associated with an up-regulated RAS, including an excess of Ang II and increased blood pressure (Y. C. Li, et al., 2004).

Angiotensin II is a pro-inflammatory agent known to exert effects upon endothelial cells that results in decreased nitric oxide, increased blood pressure, and an increase in atherosclerosis, adhesion molecules and chemokines (Dandona, Dhindsa, Ghanim, & Chaudhuri, 2007; Lavie,

Lee, & Milani, 2011). Therefore, a potential link between Vitamin D and depression is *inflammation* that occurs due to elevated Ang II and subsequent endothelial dysfunction. The link between Vitamin D level and depression has been supported in numerous studies (Ganji, et al., 2010; Hoang et al., 2011; May, et al., 2010; Tolppanen, et al., 2012) and inflammation likely poses a contributory pathway between Vitamin D and depression (Al-shair, et al., 2011; Kop, et al., 2002). Consequently, both inflammation and hypertension represent indirect, but co-related pathways (because HTN contributes to endothelial dysfunction and inflammation) that may be associated with the development of depression.

The Puglisi Vitamin D and Depression Model Summary

Evidence for a direct association between serum Vitamin D and depression appears relatively convincing. There were few studies finding no association between low serum Vitamin D levels and depression, and in fact, one likely occurred due to the younger sample utilized, most of whom had sufficient Vitamin D levels (Dean, et al., 2011). Additionally, low serum Vitamin D levels are associated with an up-regulated RAS. The model in Figure 1 proposed that low Vitamin D level may be associated with blood pressure measures and serum or endothelial measures of inflammation, which in this study will be measured by hs-CRP and endothelial dysfunction which reflects inflammation (Jablonski, et al., 2010). Thus, it is likely that both HTN and inflammation may also be associated with the development of depression.

This study will fill an important gap in the literature because it remains unclear if serum markers of inflammation are consistently higher in those who are depressed (Howren, et al., 2009). If increases in markers of inflammation are consistently associated with low Vitamin D levels, clarification of which markers vary would be useful, with future studies utilizing differing populations from persons with CAD. Also, this study clarified which type of blood pressure (SBP, DBP or HTN) was associated with low Vitamin D level, and if elevated blood pressures

were significantly associated with depression. The benefit of this research, which was based upon this new theoretical model, is that this study may establish theoretical support for future interventional trials that would not be warranted without a new understanding of how Vitamin D levels, blood pressures or HTN, serum and endothelial measures of inflammation and depression are linked. Once evidence for direct and indirect associations between Vitamin D levels and depression appear convincing, future interventional trials will be useful in establishing Vitamin D as a new low-cost, readily available agent capable of suppressing blood pressure or inflammation while perhaps preventing or treating depression. Such a finding would have major public health implications. Additionally, maintenance of the public's health in a fiscally responsible manner demands that because of the poorer outcomes of depressed persons with CAD and the potential increased platelet aggregation in those whose depression is treated with SSRIs, all potentially new therapeutics for both the *prevention* (not just the treatment) of depression should be explored.

CHAPTER III

METHODS

Introduction

This chapter describes the methodology used to evaluate the associations among demographic factors, serum Vitamin D levels, measures of HTN ([HTN], systolic and diastolic blood pressures), serum and endothelial measures of inflammation, and depression. Included are narratives detailing the research design, the sample and setting, protection of human subjects, procedures, the data analyses plan and strengths and limitations to the study.

Design

The design of this study is associational because only one group, CAD patients, is employed (Gliner, Morgan, & Leech, 2009), and the study utilizes a cross-sectional design. Non-experimental, cross-sectional designs have data collected at one point in time and do not employ an active independent variable (IV) such as Vitamin D supplementation. Cross-sectional designs provide a snapshot of the criteria at a given point in time and are used to describe phenomena early in a research trajectory to determine relationships and make comparisons (Gliner, et al., 2009). Cross-sectional studies cannot establish cause and effect between variables and are descriptive in nature answering questions of comparison and association by use of correlations, *t* tests, analysis of variance (ANOVA), logistic and multiple regressions. Therefore, this design was appropriate for the aims of this study which explored relationships between variables and group comparisons (such as men versus women, by race, and those with and without depression) to clarify the association of Vitamin D levels with these demographic and other variables. This study involved new data obtained from serum that was previously frozen in the primary study to

determine serum Vitamin D levels and liver function. Additionally, this study was a secondary analysis of a primary study designed to explain the relationship between digital peripheral arterial tonometry (PAT) and brachial artery flow mediated dilation (BAFMD) in basal and reactive hyperemic evoked conditions in persons with coronary artery disease (CAD) (Lee, et al., 2012). Specifically, this study compared findings in those with and without depression to examine the association of demographic factors upon Vitamin D levels, and the association of Vitamin D levels with HTN, BAFMD, reactive hyperemia index (RHI), augmentation index (AI) and high-sensitivity C-reactive protein (hs-CRP), and depression. This approach was also deemed suitable for the study because the independent variables (Ivs) were attributes (age, sex, race and HTN) that could not be manipulated. Further, various quantitatively measured physiologic attributes (body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), hs-CRP, BAFMD, RHI, and AI) cannot be ethically manipulated through the study protocol. Additionally, findings could not be influenced due to the use of careful inclusion/exclusion criteria. Thus, it is logistically impossible in the case of the attributes, and unlikely in the case of the quantitative variables used within the current design, to manipulate these variables to influence their one-time measurement.

Relationships in models can be associational, causal, direct, indirect, moderating, mediating or reciprocal. Moderators explain under what conditions a predictor (IV) influences the dependent variable (DV). Moderator variables can be nominal, interval, continuous or ratio level and are tested by multiple regression, and/or structural equation modeling (Mertler & Vannatta, 2013). Typically, moderators exist when the relationship between the DV and IV is strong. Moderators can also be considered at work when an unexpectedly weak or inconsistent relationship occurs between the IV and DV, because they influenced that relationship, and the moderator may change the direction of the relationship of the IV and DV (Kim, Kaye, & Wright,

2001). On the other hand, mediators may be variable(s) or mechanism(s) by which an IV influences a DV. Mediators explain the why and how relationships exist between the predictor and the DV (Kim, et al., 2001). There is usually a strong relationship between a DV and an IV before one examines for mediating effects. Examples from the proposed theory of possible physiologic mediators are the indirect or mediating effects of both HTN and inflammation upon depression separately from how Vitamin D levels and demographic variables may directly influence depression.

Setting

The primary study was conducted in Orange County, Chapel Hill, North Carolina after recruitment of subjects from The University of North Carolina (UNC) Cardiac Catheterization Laboratory. Orange County, North Carolina is a county that is 77.5% White, 12.4% Black, and 8.2% Latino (U. S. Census Bureau, 2013b). Based on these Orange County racial demographics and despite the state population being 22% Black in 2010 (U.S. Census Bureau, 2013), the original convenience sample of Lee et al. (2012) under-sampled Blacks because they represented 18% of subjects in both the coronary artery disease (CAD) and healthy volunteer groups. Also, because the local population demographics show 8% Latinos, and only one was in the sample, this group was under-sampled in the primary study (Lee, et al., 2012). Thus, no analyses were done by ethnicity. This is a weakness that may affect the generalizability of the findings because Latinos are even more prevalent (than the 8.1% of Orange County) in counties that surround Chapel Hill area from which cardiac patients are likely referred to UNC Hospitals. However, it is possible to rebut the under-sampling of Latinos with the knowledge that the mean age of Latinos in NC (24) is lower than that of the general population, making Latinos less likely to have CAD (Guitierrez-Gunter & Hall, 2012).

Sample

The sample for this study was a sample of 101 patients who had complete measures and frozen alliquoted blood available from the original 111 CAD patients. Inclusion criteria of the primary study were age ≥ 18 years of age, ability to speak and read English, and angiographically confirmed CAD which was defined as ≥50% stenosis in at least one major epicardial coronary artery by coronary angiography (Theken, et al., 2012). Exclusion criteria were: (a) pregnancy; (b) acute coronary syndrome or ischemic stroke in the previous 3 months; (c) current unstable angina (chest pain at rest); (d) atrial fibrillation, an implanted pacemaker or defibrillator, SBP > 160 or < 90 millimeters of mercury (mm Hg), DBP > 100 mm Hg, history of left-ventricular systolic dysfunction (ejection fraction ≤ 35%); € severe aortic stenosis or idiopathic hypertrophic sub-aortic stenosis; (f) upper extremities with known vascular obstruction; (g) dementia; (h) current use of long-acting nitrates or insulin; (i) active autoimmune disease; (j) history of solid organ transplant or dialysis; (k) history of HIV; (l) history of hepatitis B or C infection or fatty liver disease; (m) history of tuberculosis or high risk for tuberculosis; (n) skin diseases precluding use of electrocardiogram electrodes; (o) hypersensitivity to nitroglycerin or other nitrates; (p) respiratory infection in the preceding 4 weeks; or (q) history of cancer within the previous five years (Theken, et al., 2012). Subjects had to be willing to fast overnight and withhold all morning medications (including anti-depressants) until after their study visit and not exercise the morning of the study visit. Additionally, subjects had to agree to withhold all anti-oxidants (Vitamins C and E), fish oil, niacin, and arginine as well as vasoactive agents such as decongestants, erectile dysfunction drugs, and anti-inflammatory medications except for low dose aspirin.

Power and Sample Size Considerations

Specific aims were analyzed with logistic or various linear regressions. For the logistic regressions to model depression as the DV utilizing a two-sided Type I error level of 0.05, and 80% power with up to 6 independent predictor variables similar to that posed within research questions (3-RQ1 and 3-RQ2), the minimum sample size is 70 patients (see Table 1).

Investigating HTN requires the understanding that at age 45-54, HTN is present in 38% of men and 34% of women, becomes equal in prevalence at 52% in both sexes at age 55-64, but after age 65, HTN is present in more women (71%) than in men (64%) (Writing Group for the American Heart Association, 2013). The sample from which this data was drawn had a mean age of 59 for the CAD patients. Thus, using the above statistics, HTN was likely present in approximately 52%, or perhaps more, due to all subjects having CAD (for which HTN is a risk factor) (Lee, et al., 2012). Consequently, per nQuery Advisor (Boston, MA) if 52% of the sample has HTN, then an odds ratio (OR) of 0.46 for low Vitamin D levels on HTN can be detected with at least 80% power at a sample size of 70 when adjusting for age, sex, race, ethnicity and BMI assuming a 0.05 Type 1 error rate (Elashoff, 2007).

Table 1

Power Calculation for Vitamin D Levels on Hypertension (N = 70)

HTN Prevalence	5% HTN	10% HTN	33% HTN	50% HTN	52% HTN	75% HTN
Power	≥ .80	≥ .80	≥ .80	≥ .80	≥ .80	≥ .80
Type 1 error	.05	.05	.05	.05	.05	.05
Effect size for Odds Ratio	.26	.33	.44	.46	.46	.42

Note: HTN = Hypertension; decimals rounded up at 0.5%

Because the observed proportion of depression is unknown, the prevalence will be estimated based upon the literature. Depression is present in 6.7% of the adult population of the United States during any 12- month timeframe (National Institutes of Mental Health, n.d.). The conceptual model denotes that HTN and endothelial dysfunction, both CAD precursors, precede depression. The sample for this study utilizes only persons with CAD, so depression's prevalence in CAD patients was sought because it was expected to vary from the general population due to the chronicity of CAD. Depression has been found to have a 9.3% 12-month prevalence in 31,000 outpatient cardiac patients (Egede, 2007), but it has been debated if the depression precedes or follows the CAD. Other studies have shown depression present in 15-20% of persons with various heart conditions (Pozuelo, et al., 2009). A review of the literature on depression in CAD found depression was present in 17-44% of CAD patients (Khawaja, et al., 2009), and another study found the condition present in 25-30% of persons with CAD (Rivelli & Jiang, 2007). Thus, taking a mean of the above estimates that were specific for depression in persons with CAD (30% and 17-44%), a 30% prevalence was assumed (Khawaja et al., 2009; Rivelli & Jiang, 2007). It is not known if this percentage would be different for persons recently diagnosed with CAD or inpatient versus outpatient CAD subjects. This analysis only involved an outpatient sample of persons with CAD. Thus, a wide range of scenarios for power analysis were considered and are summarized in Table 2 according to various percentage prevalences of depression. For example from Table 2, if 30% of the sample of CAD patients had a diagnosis of depression, then an odds ratio (OR) of 0.43 for Vitamin D levels on depression can be detected with at least 80% power when the sample size is 70 and is adjusted for age, sex, race, ethnicity, BMI, HTN measures (using either SBP and DBP or HTN), and one of the serum and endothelial measures of inflammation assuming a .05 Type I error rate.

Table 2

Power Calculation for Vitamin D Levels on Depression (N = 70)

DEP Prevalence	5% DEP	10% DEP	15% DEP	20% DEP	25% DEP	30% DEP
Power	≥ .80	≥ .80	≥ .80	≥ .80	≥ .80	≥ .80
Type 1 error	.05	.05	.05	.05	.05	.05
Effect size for Odds Ratio	.26	.33	.37	.34	.42	.43

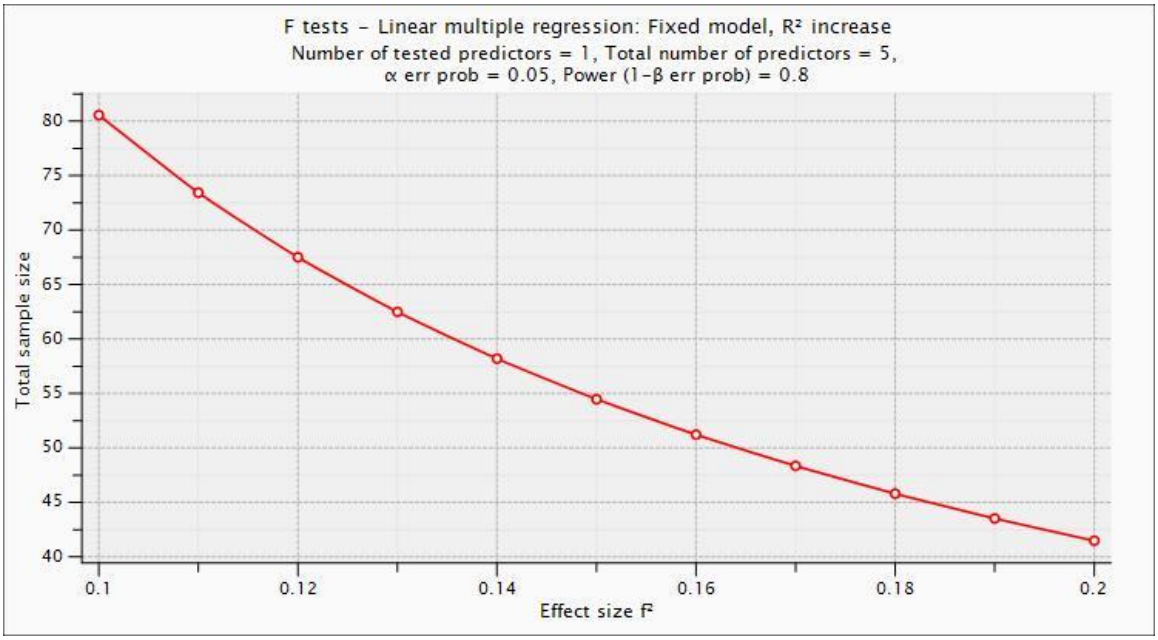
Note: DEP = depression; decimals rounded up at 0.5%

The sample size calculation herein was not powered for the more complex research questions such as Question 3-RQ3 that has potentially 13 Ivs in the model. Calculating a sample size for such a question would have precluded utilizing this secondary data set. This is because even when rules are relaxed, it is typical to require at least 5-9 events per variable within a logistic regression (Vittinghoff & McCulloch, 2007). Based upon the proposed physiologic model, calculations for sample size were focused more upon the Vitamin D levels indirect association via the continuous variables of blood pressures and serum or endothelial measures of inflammation, as well as for the presumed direct association of Vitamin D levels upon depression (see Figure 1). This was deemed reasonable because for the physiologic model to appear plausible, it would be minimally necessary for there to be a relationship herein between Vitamin D levels and HTN and/or Vitamin D level and serum or endothelial measures of inflammation prior to asserting that Vitamin D level has indirect effects upon depression. Thus, in examining the necessary power and sample size for question 3-RQ2 including inflammation, with each inflammatory measure as a DV assuming a 0.05 Type 1 error rate, a small effect size of 0.15 (Cohen's $d^2 = 0.15$) can be detected with at least 80% power when there are seven total predictors (age, sex, race, BMI, Vitamin D levels and another predictor) with a sample of 55. A smaller

significant effect size of 0.11 (Cohen's d^2 = 0.11) is detectable with a sample of 70, and with 101 subjects an effect size of < 0.10 is detectable (see Figure 2). The researcher chose to use this larger sample of 101 to utilize the total sample of CAD subjects with full serum and endothelial measures of inflammation from the primary study to allow for an even smaller effect sizes to be detectable.

Figure 2

nQuery Power Analysis for the Effect of Predictors upon Inflammation or Hypertension



Human Subjects

The study was initially approved by The University of North Carolina (UNC) Biomedical Institutional Review Board, and approval was obtained for this secondary analysis by the Institutional Review Board (IRB) of UNC, Greensboro. In accordance with standard measures to protect human subjects, consent was obtained to assure that the Belmont principles of respect for person, beneficence and justice were upheld (U. S. Department of Health and Human Services,

Office of Human Research Protections, 1979). Even though Orange County has more high school graduates than the state average (90% versus 84%) and many more Bachelor's degrees than typical for the state (54.6% versus 26.5%), the county also has more foreign born persons than the state average (13.4% versus 7.4%). Thus, it is possible that a consent form written only in English (at the 8th grade level) may have affected participant characteristics, especially inclusion of first generation immigrants residing in close proximity to UNC-Chapel Hill Hospital. The consent form included language that participation was voluntary, and withdrawal was possible without penalty from the study at any time. The study's code book was kept in a password protected file that was data encrypted by Truecrypt to ensure confidentiality, and any data not kept encrypted or password protected were de-identified data. Alliquoted vials of blood collected from the primary study had unique identification numbers assigned to them that matched subject numbers that were recorded within a code book that was kept in a secure location.

Instruments

There were no paper instruments utilized in the primary study, only physiologic measures which were recorded in a password protected Microsoft Excel (2010) file.

Procedures

Between October, 2007 and December, 2010 a sample of 101 CAD subjects was enrolled in the original study that included both 41 healthy volunteers (not utilized herein) and 111 persons with CAD (of which 101 had complete data). Subjects with CAD who were clinically stable (no chest pain at rest) returned within 3 months of recruitment from a cardiac catheterization laboratory (61 days ± 30) to the clinical research unit (Lee, et al., 2012). Participants had performed an overnight fast. After patients arrived at the outpatient clinic research unit, trained clinical research assistants obtained informed consent. Subsequently,

subjects were placed on a bed with the sphygmomanometer on the subject's right forearm, and the EndoPAT probes were placed on the fingertips of both hands. Following 10 minutes of rest in the supine position, subjects' baseline blood pressures were measured. The EndoPAT tracing was started while the sole experienced ultrasound sonographer simultaneously obtained the baseline brachial artery diameter readings. When these procedures were complete, the sphygmomanometer was next inflated to 70 mm Hg above the subject's previous SBP for 5 minutes. Occlusion of blood flow was confirmed by the EndoPAT during this time. Following release of the blood pressure cuff, the EndoPAT tracing was recorded while the sonographer simultaneously obtained the post-hyperemic brachial artery diameter readings. Following 10 minutes of rest, the sonographer obtained a second baseline brachial artery diameter measurement. The subject was then given a 0.4 mg sublingual nitroglycerin tablet, and the sonographer measured the brachial artery diameter 5 minutes later to assess endothelium-independent vasodilation. Following 10 additional minutes of rest and confirmation that subjects were not hypotensive, subjects were walked to hematology where their blood and urine were collected. Subjects were given a snack and beverage before they left, a $50 incentive, a validated parking pass and thanked. The total time for each research visit was approximately 60 minutes.

Full detailed procedures have been published for the collection of the endothelial measures, but only the variables utilized within this secondary analysis are described herein (Lee, et al., 2012). Brachial artery diameter was calculated as the difference at the end of diastole between the proximal and distal walls in the supine position. In the present study (Lee et al., 2012) the brachial artery diameter was averaged from 10 consecutive frames and was assessed 90 seconds after the blood pressure was deflated. Then, BAFMD was calculated as the percentage change in the brachial artery diameter relative to baseline changed into percentage, BAFMD = 100 x ($diameter_{peak} - diameter_{baseline}$/ $diameter_{baseline}$). The BAFMD measures were analyzed

utilizing Brachial Tools software (Medical Imaging Applications, Coralville, Iowa; (Lee, et al., 2012), and the procedure was performed as has been previously published within the literature (Benjamin et al., 2004; Corretti, et al., 2002).

EndoPAT 2000 (Itamar Medical) is a Food and Drug Administration approved device indicated to establish endothelial dysfunction utilizing reactive hyperemia, and EndoPAT was utilized to calculate both RHI and AI. Augmentation index is a surrogate marker for arterial stiffness associated with both low serum Vitamin D and endothelial dysfunction (Al Mheid, et al., 2011; Lee, et al., 2012). The EndoPAT obtained pulse volume amplitude on the index finger of each hand with plethysmographic probes making continuous measures during the time the brachial artery measurements were being taken. Measures obtained at baseline, during 5 minutes of cuff occlusion and during the 5 minutes post-cuff deflation were utilized by the EndoPAT software version 3.04 to calculate the PAT, RHI and the AI (Lee, et al., 2012). Because there were a few error readings during the use of the EndoPAT, data from 10 of the original study's 111 CAD patients was excluded (Lee, et al., 2012). The procedures utilized are consistent with prior publications (Hamburg et al., 2009; Lee, et al., 2012).

Within the primary data study, the serum of the 111 CAD subjects had been stored after blood samples were initially collected between October, 2007 and December, 2010. The serum was centrifuged and then frozen to -80̊ C in alliquoted samples stored in the freezer of Dr. Craig Lee's clinical research lab at UNC, Chapel Hill. To assure specimen integrity, the frozen serum was transported by a Labcorp courier on dry ice to their lab in Burlington, North Carolina; serum stability is accurate for Vitamin D levels and liver function tests after one freeze-thaw cycle (L. Wideman, personal communication, July, 2013). Labcorp performed the testing for the current study's data collection which consisted of serum Vitamin D levels [25(OH)D], aspartate aminotransferase (AST) and alanine aminotransferase (ALT) analyses. Labcorp is a CLIA-

certified laboratory (Clinical Laboratory Improvement Amendments) that frequently performs Vitamin D and AST and ALT analyses (U.S. Centers for Medicare and Medicaid Services, 2006). Labcorp's method of completing the AST and ALT level is spectrophotometry. Labcorp uses an immunochemiluminometric assay performed on the DiaSorin LIAISON® analyzer (Saluggia, Vercelli, Italy) to calculate the Vitamin D levels, and this automated test measures both serum Vitamin D_2 and D_3 as a total number, as is the custom in the United States. According to Labcorp company literature, many large clinical studies have utilized DiaSorin testing for Vitamin D levels, including: National Health and Nutrition Examination Survey (NHANES), the Women's Health Initiative (WHI) Studies, and the Harvard-Based Health Professionals Studies.

Data files of de-identified data were provided to the student investigator by Dr. Craig Lee of the UNC, Chapel Hill. The data was in a Microsoft Excel (2010) file that was password protected when created by Dr. Craig Lee.

Data Analyses

Original analyses by Dr. Craig Lee were performed using SAS, version 9.2 (SAS Institute, Cary, North Carolina) with an alpha set at $p \leq 0.05$ (Lee, et al., 2012). The student transferred this data from a Microsoft Excel (2010) spreadsheet into Statistical Packages for the Social Sciences (SPSS), Version 20 (International Business Machines Corp, 2012).

Descriptive statistics with frequencies for all nominal and ordinal variables were calculated. For continuous variables, the mean, median, range, kurtosis and skew were calculated. Interval level data that was not normally distributed were transformed as needed to meet assumptions of the various statistical tests. Interval level predictor variables (Vitamin D levels, blood pressure, hs-CRP, BAMFD, RHI and AI) were assessed first by boxplot, and outliers were noted. Then, combinations of variables were assessed by scatterplot to check the linearity of their relationship(s). Calculations were performed with and without outliers because

outliers skew correlations and means, and can also make regressions unstable. Normality was assessed by the Kolmogorov-Smirnov (K-S) test first so that correlations of variables with non-normal distributions could be performed by Spearman's *rho* rank-correlation instead of a Pearson's *r* correlation. To help assure internal validity, equivalence of groups was assessed by comparing the mean age and BMI, and the proportion of sex and race in those with normal and abnormal Vitamin D, and with and without depression. Additionally, the number of subjects with a hs-CRP > 10 mg/dL was recorded, and their outcomes examined separately because a hs-CRP > 10 mg/dL often means an acute process, such as trauma or infection, is ongoing (Ridker, 2003).

Bivariate correlations were assessed between continuous variables to ascertain if results follow the strength and direction of predictions from the literature. A positive correlation is expected for measures of inflammation with increasing age (Wilkerson & Sane, 2002), for increasing age and blood pressure, and for decreased glomerular filtration rate and decreased Vitamin D (American Heart Association Statistics Committee and Stroke Statistics Committee, 2012; Cronin, 2010). A strong negative correlation is expected for increased blood pressures and measures reflecting decreased endothelial function (Jablonski, et al., 2010), as well as a negative correlation being expected for increased liver function and decreased Vitamin D (Putz-Bankuti et al., 2012). An inverse correlation between SBP and serum Vitamin D levels was expected (A. Fraser, et al., 2010; A. K. Gupta, et al., 2011; R. Scragg, et al., 2010). The correlation of angiotensin-converting enzyme inhibitor or angiotensin receptor blocker use and depression is expected to reflect an inverse association because angiotensin II is decreased in individuals who use these HTN agents (Saavedra, Sanchez-Lemus, & Benicky, 2011). There is lack of clarity about how sex (male and female) affects Vitamin D levels. One study found it lower in men (Hagenau et al., 2009), but it may be lower in women due to women typically having less time

outside (Ginde, et al., 2009; McGreevy & Williams, 2011). Thus, the correlation between sex and Vitamin D status was also assessed to determine its strength and direction.

For the specific aims addressing the association of Vitamin D, blood pressures, and serum and endothelial measures of inflammation with depression, the researcher chose to follow the tradition of multiple previous research studies and performed analyses with interval level data and collapsed tertiles. Vitamin D level data were collapsed into three categories: sufficient, insufficient and deficient. These collapsed values classified Vitamin D levels as sufficient when $\geq$ 30 ng/mL, insufficient when between 21-29 ng/mL, and deficient when $\leq$ 20 ng/mL (Holick, et al., 2011). Tertiles of Vitamin D level sufficiency status were used when running the regressions for research questions 2-RQ1, 3-RQ1 and 3-RQ2 utilizing the logistic regression and multiple linear regressions as is appropriate for the DV. Utilizing collapsed tertiles of Vitamin D levels was deemed appropriate because the specific aim for these questions was to explore potential relationships between Vitamin D levels, HTN, serum and endothelial measures of inflammation and depression. Additionally, breaking Vitamin D levels into tertiles was a good idea for Specific Aim 3 because published studies on Vitamin D levels have broken the variable into these tertiles which will allow for a better comparison of results across studies.

Data Analyses for Specific Aims

Next follows a discussion of the three specific aims and the data analysis for each aim.

1. Describe the established stable CAD population's Vitamin D levels, HTN measures and serum and endothelial measures of inflammation in the depressed and non-depressed.

    a. 1-RQ1: What proportion of the sample is depressed?

        The percentage of the sample on and not on an antidepressant was calculated along with their 95% confidence intervals.

b. 1-RQ2: What are the mean Vitamin D levels, serum hs-CRP level, BAFMD, RHI, and AI in those who are and are not depressed?

The sample was split into those with and without depression. Then, descriptive statistics for continuous variables, including the mean, standard deviation, range, skew, kurtosis and 95% confidence intervals (CI) were calculated for serum Vitamin D levels, hs-CRP, BAFMD, RHI and AI, as well as for age and BMI.

c. 1-RQ3: What proportion (%) of the sample has systolic or diastolic HTN?

The percentage was calculated for those with SBP $\geq$ 140 mm Hg, and/or DBP $\geq$ 90 mm Hg and those deemed to have a medical diagnosis of HTN along with their 95% CI for HTN.

d. 1-RQ4: Is there a difference in Vitamin D level, SBP, DBP, HTN, BAFMD, RHI, and AI in those who are and are not depressed?

The appropriate analysis for comparing group differences with a categorical variable (depression or not) and two or more mixed predictor variables is individual *t* tests for comparing the means of the groups (Mertler & Vannatta, 2013). Therefore, this question was initially planned to be analyzed by six independent sample *t* tests comparing the mean serum Vitamin D, SBP, DBP, BAFMD, RHI and AI in depressed and not depressed subjects. Assumptions were first checked for the independent sample *t* test which include independent observations, normality of data (assessed by the K-S test), no extreme outliers and equal variances (Huck, 2008). The alternate to the *t*-test when normality is in question, extreme outliers are present or there are non-equal variances is the nonparametric Mann-Whitney

U test. The analyses for this study utilized the Mann-Whitney U test when normality was in question (Huck, 2008). Non-equal variances were assessed by Levene's test for equal variances which yields a non-significant *p* value when equal variances, is assured, and SPSS output allows interpretation based upon either equal or non-equal variances.

When variables are binary, such as in HTN and depression, the research question was answered by the Chi-Square. Thus, Chi-Square was used to determine if the presence of HTN differed in those on and not on an anti-depressant. Chi-Square tests a theoretical distribution where events are mutually exclusive and have a total probability of 1. Like all statistical tests, Chi-Square has assumptions which were assured prior to utilizing this test, including: (a) an adequate sample size with a common rule that expected cell sizes in a 2x2 contingency table are at least 5, and (b) independent observations (Huck, 2008). When assumptions are not satisfied for the Chi-Square test, then Fisher's exact test was utilized, and SPSS conducts this analysis with a warning to not utilize Chi-Square when less than 5 occurrences are present per cell.

2. Explain the relationship of Vitamin D to depression.

   a. 2-RQ1: Is Vitamin D level associated with depression?

The state of having depression or not was re-coded into a binary categorical variable wherein 1= depression and 0 = no depression to facilitate performing a logistic regression (Huck, 2008). Within SPSS, two binary regressions were performed: (a) the first with Vitamin D as a continuous variable, and (b) the second with Vitamin D grouped into tertiles (sufficient

Vitamin D being ≥ 30 ng/mL, insufficient between 21-29 ng/mL, and deficient ≤ 20 ng/mL (Holick, et al., 2011).

A benefit of performing logistic regression is that it does not require assumptions about the distribution of the predictor variables, which is an assumption of multiple regression. Logistic regressions were performed to explain the association of serum Vitamin D levels (the IV) with depression after the assumptions of logistic regression were first checked. These assumptions include: (a) one DV which is dichotomous (depression), (b) independence of measures (which this design assures), (c) no important predictor variables omitted, (d) no extraneous variables included, and € the IV is measured without error (Huck, 2008; Mertler & Vannatta, 2013).

Typically in a logistic regression, because of the assumption that no important predictor variables are omitted, testing the purity of the relationship requires some control of variables. Thus, when confounding is expected by variables known to affect a predictor variable or outcome (such as the effect of age upon Vitamin D level), one controls for such confounders, like age, herein. The need to control for confounders affecting the DVs is the rationale for questions 2-RQ2, 3-RQ4 and 3-RQ5 below (Huck, 2008).

b. 2-RQ2: When controlling for age, sex, race and BMI, do Vitamin D levels differ in those with and without depression?

To answer this question, as in 2-RQ1, logistic regression was used once assumptions were met. The researcher entered age, sex, race and BMI as Ivs within the first Block in SPSS, and Vitamin D in Block 2. Because the

purpose of this question was exploratory and testing the associations found in a model (Figure 1), the forward stepping method of entering the multiple Ivs (age, sex, race, BMI and Vitamin D) was utilized so that only Ivs that were significantly associated with the DV would be in the final model. The demographic variables were considered separately from the effect of Vitamin D levels. The output from SPSS showed a Chi-square goodness of fit that determines if these Ivs should remain in the model. Only Ivs found to be significant ($p < 0.05$) were included in the model to explain depression.

3. Examine the relationship of Vitamin D levels, HTN measures (SBP, DBP, yes/no HTN) and serum and endothelial measures of inflammation in those who are and are not depressed.

   a. 3-RQ1: What is relationship of Vitamin D levels to measures of HTN (SBP, DBP, yes/no HTN)?

      This question was answered by two different statistics because HTN is a binary variable, while SBP and DBP are continuous variables. HTN was re-coded numerically into 1 = hypertension, 0 = no HTN, as per a diagnosis within the medical record. Then, similar to the analyses in 2-RQ1, logistic regressions were performed to assess the effect of Vitamin D upon HTN's prevalence. Before running this analysis, assumptions were checked as previously reviewed in question 2-RQ1 above.

      Next a multivariate multiple regression modeling of SBP and DBP was performed, and if either SBP or DBP was significant, this provided a rationale for performing simple linear regressions to check for the contribution of Vitamin D upon blood pressures. The DVs entered were SBP

and DBP, with a covariate of Vitamin D within SPSS, and this output from the Analyze →General Linear Model tab in SPSS also yields the individual simple linear regressions for SBP and DBP which were examined when the multivariate model was significant. The significance value found for Vitamin D's effect on SBP and DBP in the multivariate model was the Wilk's Lambda value, which when not significant, means that the Vitamin D levels were not associated with either SBP or DBP.

Multivariate models are more powerful than simple linear regressions examining the effect of Vitamin D upon SBP and DBP because this analysis reduces unexplained variance. Both multivariate multiple regression and separate multiple regressions require meeting assumptions which are: (a) linearity between the IV and DV (assessed by scatterplot), (b) normality of residuals (the Studentized deleted residuals), (c) independence of errors (utilizing the Durbin-Watson statistic) and independent observations, (d) homoscedasticity (error variance is constant and assured by the Levene's test which yields a non-significant *p* value when homoscedasticity is assured), € appropriate variables in the model that add to prediction, (f) no high correlations > 0.80 reflective of multicollinearity between Ivs (Allison, 2007), and, (g) extreme outliers are limited because regression models are sensitive to outliers (Mertler & Vannatta, 2013).

Linearity between the Ivs was assessed by bivariate scatterplots for each IV and SBP and DBP. Normality of residuals was assessed by looking at the skewness and kurtosis, which are zero when variables are normally distributed (Mertler & Vannatta, 2013). Normality was assessed by both P-P

plots or a one sample Komolgorov-Smirnov (K-S) test for each variable which yields a non-significant *p* value when the variable is normally distributed. This sample and study design had observations which were independent of each other. Multicollinearity was checked by a search for influential points, or outliers, by variance inflation factors (VIFs), Cook's Distance and Df-BETA. When the VIF of a predictor is greater than 10, this is a cause for concern (Mertler & Vannatta, 2013). Cook's Distance was determined and represents the general influence of a data point within the regression equation by deleting the nth observation with a value of 1 or more indicating an influential point. Df-BETA was also calculated for each observation to show the researcher if the regression coefficients change from one observation. When Df-BETA has a value ≥ 2 or more than $2\sqrt{101}$, this indicates an extreme point that has influence. Thus, when influential points were found in various variables (particularly within Vitamin D herein), the regression findings were reported with and without these observations, drawing attention to whether the one influential point changed the findings.

If SBP or DBP is significant within the multivariate multiple regression, each significant IV in the aforementioned multivariate regression was then analyzed within individual simple linear regression(s) with serum Vitamin D level as the IV. Assumptions of simple linear regression are similar to those of multiple linear regression with the exception of there being no concern with multicollinearity because in simple regression there is only one IV, so no further assumption checking is required for these simple linear analyses.

b. 3-RQ2: What is relationship of Vitamin D levels to serum and endothelial measures of inflammation (hs-CRP, BAFMD, RHI, and AI)?

This question was answered similar to question 3-RQ1. Instead of SBP and DBP placed as the DVs, hs-CRP, BAFMD, RHI and AI were the DVs placed into in multivariate multiple regression. Again, Vitamin D level (continuously and in tertiles in two different regressions) was the predictor variable or covariate in this multivariate regression modeling inflammation. Assumptions were assured as described, and if Vitamin D level was significantly related to any of the four measures of inflammation, then individual simple linear regressions were performed to assess Vitamin D's association with inflammation (hs-CRP, BAFMD, RHI and AI) as described in 3-RQ1.

c. 3-RQ3: Are measures of HTN (SBP, DBP, yes/no HTN), and serum and endothelial measures of inflammation (hs-CRP, BAFMD, RHI, and AI) associated with the occurrence of depression?

As in 2-RQ1 above, one logistic regression was performed utilizing SBP, DBP, HTN, hs-CRP, BAFMD, RHI and AI as predictors to assess for a significant affect upon group membership—depressed or not depressed. When these Ivs were entered into the blocks in SPSS, HTN was recoded as 1 when present, and 0 when not. Then, Block 1 was entered to include the three hypertension variables, and Block 2 contained the four measures of inflammation associated with depression in a forward stepping regression so that only significant variables would be carried forward. Assumptions were checked for logistic regression as per question 2-RQ1 above.

d. 3-RQ4: When controlling for age, sex, race and BMI, are Vitamin D levels, serum and endothelial measures of inflammation, (hs-CRP, BAFMD, RHI and AI) and measures of HTN (SBP, DBP and HTN) associated with depression?

This question was again answered by logistic regression because depression is binary, and this questions was conducted similarly to 2-RQ2 above. Because this is also an exploratory question, the forward stepping method was again be used to enter into the model the multiple Ivs (age, sex, race, and BMI), so that only Ivs that predict the DV in a significant manner were utilized within the final model. When entering these variables into the logistic regression in SPSS, the demographic variables were entered into Block 1, blood pressures into Block 2, Vitamin D levels into Block 3, and measures of inflammation (hs-CRP, BAFMD, RHI and AI) into Block 4. This method of entering within the blocks was chosen because the primary interest of the researcher in this question is with the effect of Vitamin D and inflammation upon depression, so these variables were entered into the later blocks. It was expected that age and BMI may influence inflammation, and the researcher proposed from prior research findings that perhaps blood pressure and race may affect Vitamin D. Assumptions were assured as previously described for logistic regression in 2-RQ1. However, special attention was given to the relationships between Vitamin D levels and blood pressure measures and Vitamin D levels and inflammation, assessing for multicollinearity because a significant relationship may have been found in the analysis of earlier questions. To assess for issues with multicollinearity, a multiple linear regression was conducted with depression as the DV

utilizing the demographic variables, measures of HTN and measures of inflammation as predictors to show the VIFs, so that results were given with an without any influential points found within the VIFs.

e. 3-RQ5: When controlling for age, sex, race, BMI and Vitamin D levels, are serum and endothelial measures of inflammation and measures of HTN associated with depression?

This question was again answered by logistic regression and assumptions of logistic regression were checked as previously described in 2-RQ1. Additionally, if in 2-RQ1 there was a significant association between Vitamin D levels (either as continuous or tertile variables) and depression, this Vitamin D level variable was to be added into the block in the final model that showed the relationship between HTN, inflammation and depression.

As in 3-RQ4, because this is an exploratory question, the forward stepping method was used to enter into the model the multiple Ivs (age, sex, race, BMI and Vitamin D levels) so that only variables that predict the DV in a significant manner were retained within the final model. When entering these variables into the logistic regression in SPSS, the demographic variables and Vitamin D levels were entered into Block 1, measures of blood pressures into Block 2, and measures of inflammation (hs-CRP, BAFMD, RHI and AI) into Block 3 to show their association with depression. Assumptions were assured as previously described for logistic regression in 2-RQ1. Again, as in 3-RQ4, special attention was given to the possibility of multicollinearity between Vitamin D levels and blood pressure measures

and/or Vitamin D levels and measures of inflammation because these may have been found in earlier questions to be related.

Limitations

Scientific research that is experimental with an active intervention maximizes internal validity and provides enhanced ability to generalize findings (Walker, 2005). However, exploratory and correlational research initially lays the groundwork to justify the time and expense a researcher would expend in performing interventional studies which hold a risk of exposing participants to potentially harmful interventions. Consequently, this cross-sectional research design was chosen due to the early state of the science explaining the associations of Vitamin D levels, blood pressure and serum and endothelial measures of inflammation with depression. A weakness of the cross-sectional design is that it limits responses to one point in time. Admittedly a one-time measure may not be the best for evaluations of mood such as depression because it is known from research with the PHQ-9 that reliability at 48 hours was 0.84, but merely 0.59 a week later evidencing that depression is a highly dynamic state (Kroenke, Spitzer, & Williams, 2001; Monahan et al., 2007). Finally, cross sectional designs never establish a cause and effect relationship regardless of the strength of the positive or negative correlation that is found, nor can the design measure changes of variables over-time due to the one-time measures involved.

Recording the DV as those with a medical diagnosis of depression is both a strength and a weakness for the design. The use of an antidepressant might make a more stable and reliable predictor of longer term depression, but this binary variable could also be unreliable if the patient were on an anti-depressant and no longer clinically depressed with perhaps a symptom score ranking on a standardized tool in the non-depressed range. So while established, current depression measured by a tool may be more reliable than a one-time measure of depression and

yield the benefit of interval level data, because mood varies widely in short timespans, clinicians typically establish depression by symptoms that have been consistently present for at least two weeks (Kessler, et al., 2005). However, this key variable of depression relies upon subjects reporting their anti-depressant usage so that the medical record reliably discloses depression within the participant. These factors represent a weakness because social desirability may preclude individuals from reporting antidepressant use, particularly if they fear their cardiologist or internist will not evaluate their other complaints (such as chest pain) in an unbiased fashion due to their depression. Also, many patients in the sample of 101 were transferred into the UNC Hospitals cardiology service during an acute event, and it is possible some medications were not recorded during this transfer of care. Additionally, there is a chance that some patients may have been clinically depressed and not diagnosed and treated. Therefore, counting a depression diagnosis within the medical record as a marker for actual ongoing depression could conceivably over-estimate the true occurrence of depression in the sample, while social desirability issues may contribute to an under-estimation of the prevalence of depression. Utilizing a medical diagnosis of depression as an indicator of depression appeared most stable and reliable and was utilized. Another limitation regarding depression's prevalence in this sample of persons with CAD is that many of these subjects may be taking angiotensin-converting enzyme inhibitors or angiotensin receptor blockers which are expected in those with a history of prior CAD, and these drugs might exert a beneficial effect in lowering brain Ang II and boosting mood which would lessen an ability to detect findings in the research questions where depression is the DV (Saavedra, Sanchez-Lemus, & Benicky, 2011).

Control in research designs is exerted in several ways: random sampling, the choice of inclusion and exclusion criteria, use of a comparison group, precise measurement tools, blinding procedures, manipulation of the independent variable, subject matching in groups, and use of

standardized statistical tests for which all assumptions are met (Walker, 2005). Control in this study was established by careful inclusion/exclusion criteria, and the appropriate use of standardized statistical tests after careful analysis that the assumptions were met. The original researcher checked and re-checked data entry to assure its integrity, and the student principal investigator spot checked data imported from the Excel file into SPSS and the Vitamin D levels, AST and ALT levels were double entered to assure their accuracy.

External validity deals with the ability to generalize and is categorized into two types: (a) population external validity, and (b) ecological external validity (Gliner, et al., 2009). Population validity has to do with whether the participants are representative of the theoretical population. While it is possible to examine the demographic characteristics of the study sample to see if they are similar to those in the geographic area's population, this still does not assure represent-tativeness (Gliner, et al., 2009). In this study there was expected to be a difference between those accessible to the researcher and the population of central North Carolinians because this sample was less heavily weighted with minorities and women; the full sample of 41 healthy subjects and 108 CAD patients had only 33% women, 18% Blacks and just one Latino (Lee, et al., 2012). The data for the study from which this secondary analysis were drawn were collected from 2007-2010. The State of North Carolina in the 2010 United States Census had 51.3% women, and 22% Blacks and 8.6% Latino; thus, population external validity may be mildly weakened. However, the median ages of Latinos' makes them much less likely to have CAD, and this is evidenced within national prevalence statistics for CAD (United States Census Bureau, 2013).

Many properties of a study can lead to a non-representative sample, including: (a) convenience samples, (b) poor response rates, and, (c) attrition of participants (Gliner, et al., 2009). Attrition was not an issue in this study because the research design is cross-sectional

allowing for data collection at one point in time, although due to standard human subject's protections, participants could always ask to be withdrawn from the study after completion of data collection. There was no attrition in the primary study. The use of convenience sampling, within the original study, limited control within this research design; the sample of the original study was recruited from one hospital's cardiac catheterization laboratory. Because of the non-probability design of the primary study, the sample was likely not representative of the larger population of persons with CAD so inferences to the region's population of CAD patients (external validity) is weak, and any generalizations can only be made to a cardiac population because all individuals in this convenience sample had confirmed CAD. Despite this, there are benefits associated with convenience samples. Certainly cost and time may be saved by utilizing convenience samples. Other reasons to use convenience samples include that researchers may not be interested in making inferences about the population from which the sample was drawn but may be more interested in whether a treatment has an effect. Such researchers might deem that any effect, if powerful, will be seen in many types of participants. Regardless, a convenience sample limits external validity which means that the study is not generalizable to others measures, populations or settings, and this is a weakness herein (Gliner, et al., 2009).

Internal validity is the ability to infer a causal relationship (Gliner, et al., 2009). While traditionally this applies to randomized controlled trials and quasi-experiments, others believe that internal validity applies to non-experimental studies with attribute Ivs such as depression's prevalence (Gliner, et al., 2009). This cross-sectional study utilizes only one group of CAD patients; however, given that analyses are done that break this sample into those with various attributes such as adequate or inadequate Vitamin D levels or those over and under age 65, it is appropriate to examine internal validity issues. To assure internal validity, there are two qualities one must assess: equivalence of groups on various participant characteristics and control of

extraneous variables (Gliner, et al., 2009). Because equivalence of groups can be approximated when groups of 30 or more are used (Gliner, et al., 2009) and the power calculation for this research called for 55 subjects, it is anticipated that groups may approximate each other in this sample of 101. In the sampling design, stratifying occurred of those depressed and not depressed, so that these groups might approximate each other as to age, sex, race and BMI. It is acknowledged that groups based upon attributes are rarely close to equivalent on other characteristics, so careful data analyses were necessary when breaking the sample into groups based on various attributes (Gliner, et al., 2009). Attempts to address equivalence of groups were performed during data analyses by comparing the mean age, sex, race and BMI of those with normal and abnormal Vitamin D levels, and of those with and without depression.

The second necessary component of internal validity is control of extraneous variables. Typically this threat to validity comes into play when some participants learn the rationale for the study and change their behavior. Given that the proposed study used data collected at one point in time, this threat did not occur because of the non-experimental design. Control of the environment and experiences of the research subjects helped to assure that extraneous variables did not influence the results. The threat of extraneous variables in an associational design might occur if those who have high Vitamin D levels or another IV such as age or BMI are substantially different in those who are low on the same independent variable (Gliner, et al., 2009). Additionally, if subjects were to know the specific aims of a study, they might chose to reveal or not reveal their antidepressant usage due to some personal feeling about the disease or use of medication, and this might make the effect of Vitamin D levels appear stronger or weaker. Because in this study the data were collected for all subjects in the same type of temperature controlled, dimly lit room following the same protocol by the same examiners, the internal validity threat of extraneous variables was viewed as minimal in the primary study. Additional

internal validity issues are: (a) testing, (b) history, (c) instrumentation, (d) maturation, € mortality, and (f) selection bias (Gliner, et al., 2009). A convenience sample can result in selection bias and has been discussed. There was no attrition (mortality) in the primary study, and unlikely maturation issues since the subjects were seen during a one-hour visit. History was not an issue in this one time measure where data were collected at one point in time. Instrumentation issues might occur because of an inconsistent manner of taking blood pressures (i.e. using a manual versus automatic cuff at various times or recording the pressures based on different Korotkoff sounds), and the EndoPAT equipment was not recalibrated during the three-year study although the manufacturer did not call for this in their literature. Thus, drift in measures by the EndoPAT appeared unlikely, although a drift in blood pressures was theoretically possible.

Ambiguous temporal precedence occurring because Ivs or confounders occurred before measurement of the DV, depression, was also a risk herein. Other medications that might influence depression (beta blockers, benzodiazepines and others) were not be accounted for, nor were actual amounts and the type of Vitamin D (ergocalciferol or cholecalciferol) that participants ingested analyzed in the design. This secondary analysis also presumed that data were collected in a scientifically robust manner and that data were keyed into electronic files accurately to make them reliable and valid. In the primary study (Lee, et al., 2012), CAD was defined as ≥ 50% stenosis in one or more major epicardial arteries, and one should consider if this is an appropriate definition for a research questions seeking variables associated with depression. For instance, CAD begins in childhood, and it is unclear if lesser amounts of plaque than 50% might result in findings of depression because inflammation is involved in all steps of atherosclerosis, and inflammation has a known association with depression (Howren, et al., 2009; Kop, et al., 2002). Therefore, by using the definition of CAD being ≥ 50% stenosis in one or

more epicardial arteries, subjects with perhaps lesser CAD, due to shorter term and less severe atherosclerosis, as well as those with atherosclerosis affecting other vessels will be unknown in the study population. This would result in an underestimation of any true association between inflammation and depression**.**

Other limitations include that there was no adjustment of the serum Vitamin D levels for seasonality, even though it is known that Vitamin D level is affected by alteration in the sun's solar zenith angle (Holick, 2007). Serum samples were drawn over more than a year's timeframe in the original study which means that season may have affected serum Vitamin D values. Without adjustment for seasonality, any calculated association with depression according to some (Fraser, et al., 2010) may be an underestimation, or perhaps an over-estimation of any true association. A possible solution to this is to check the influence of the month data were collected on the serum Vitamin D levels to assess for any effect, noting that above 37 degrees latitude (at a line parallel to the North Carolina-Virginia border) because little to no Vitamin D can be produced by dermal conversion from sunlight except in the summer months (Harvard Women's Health Watch, 2008).

Another limitation is that there were very few Blacks and Latinos in this study. Because we know that Blacks have a higher prevalence of CAD (6.9% for Blacks versus 6.3% for Whites versus 5.9% for Latinos), and a larger number of deaths from CAD than Whites (387 deaths for Blacks versus 281 for Whites) despite Blacks' lesser numbers within the population at large, this reflects a sample that was not proportional to disease or demographic prevalence rates (American Heart Association Statistics Committee and Stroke Statistics Committee et al., 2012; Centers for Disease Control and Prevention, 2011a). Likely, the English-only consent form and social determinants of health, such as lower disposable income and the lack of health insurance common amongst many minorities hindered the ability to recruit minorities from a cardiac catheterization

laboratory at a well-known tertiary care center such as UNC Hospitals. Blood pressure measurements may not have been rigorously obtained by automatic sphygmomanometer in the primary study, and there is likely confounding because most CAD patients will have HTN. Of the original sample, 93% of subjects were on statin cholesterol drugs which preserve endothelial function and decrease inflammation, so this might limit significant findings in the endothelial measures but is useful for explaining group membership (Beckman & Creager, 2006; Blum & Shamburekb, 2009; Lee, et al., 2012). Thus, this study will have limitations of ambiguous temporal precedence, lack of data on subject's use of Vitamin D supplements, a lack of adjustment for seasonality in the serum Vitamin D levels, use of a convenience sample, non-rigorous blood pressure measures, some confounding variables and the limitations of a cross sectional design on a fluctuating DV, depression.

A unique strength of this study is its focus upon persons with CAD. The majority of the studies of low serum Vitamin D levels and their association with depression are in studies of a more general population, and not a cardiovascular population, although one study of a general cardiovascular population low Vitamin D levels found an association between low Vitamin D levels and incident depression (May, et al., 2010). Another strength of this study is that all subjects had CAD and were receiving optimal, state-of-the-art care; in fact, two-thirds of the 99 subjects with full measures had already had stents (55) and 10 had had bypass grafts (Lee, et al., 2012). We know that depression is a risk factor for CAD (Van der Kooy et al., 2007). Consequently, should this research find that Vitamin D, measures of inflammation, blood pressures, or the existence of HTN are significantly associated with depression, this shows another possible direction of the relationship. Depression is present in 17-44% of persons with CAD (Khawaja, et al., 2009; Rivelli & Jiang, 2007), and we know that depression within CAD is associated with poor outcomes, including heart failure (Heidi T. May et al., 2009). Even when

depression is present, identified and treated, improvement in depression has not been shown to improve cardiovascular outcomes (American College of Cardiology Foundation, et al., 2012; Glassman, et al., 2002; Writing Committee for the ENRICHD Investigators, 2003). This makes determining how Vitamin D levels, HTN, blood pressures or inflammation are associated with depression, if at all, significant. Consequently, finding significance in this study might influence future cardiovascular care for persons with CAD. Despite the noted limitations, the strengths of this proposed study and the pressing need to fill a gap in the literature about the relationships between serum Vitamin D levels, HTN, inflammation and depression justify this study's design.

Summary

This cross-sectional, associational research design sought to clarify the relationship between various demographic factors upon serum Vitamin D levels, as well as the relationship of serum Vitamin D levels, HTN, and measures of inflammation upon depression. This study's design utilized a secondary analysis of a convenience sample of all 101 adults with usable data presenting during the primary study. This study describes Vitamin D levels, blood pressures, the presence of HTN, and measures of inflammation in the sample, as well as the relationship of these variables with depression.

Data were analyzed with typical descriptive statistics, *t* tests, and logistic or multiple linear regressions to determine the relationship of demographic variables, Vitamin D levels, blood pressure measures, and serum and endothelial measure of inflammation on depression. Scrupulous attention was paid to meeting statistical assumptions and assuring that the new lab data were entered accurately into SPSS (IBM Corporation, Armonk, NY) to yield reliable and valid inferences. While the principal investigator (PI) of this secondary analysis collected none of the original data, the PI did analyze the new primary data (serum Vitamin D levels, AST and ALT levels), as well as secondary data within SPSS to answer the specific aims and research

questions. Findings clarified the relationships between variables in the model in persons with CAD and provide a useful information to assist clinicians in providing optimal care to persons with CAD.

CHAPTER IV

RESULTS

Introduction

This chapter details the results of the statistical analyses to answer the research questions. The components of this chapter include: (a) a description of the sample, (b) preliminary examination of the data set, and, (c) the rationale for the analysis conducted and the results that answer each specific research question.

Sample

Participants were recruited between October, 2007 and December, 2010 from the cardiac catheterization laboratory at The University of North Carolina (UNC), Chapel Hill. Convenience sampling was utilized to recruit persons found to have coronary artery disease (CAD) with a stenosis of equal to or greater than 50% in any epicardial artery as identified by cardiac catheterization. A total of 111 persons with CAD were recruited for the primary study, of which 101 subjects had complete data in this cross-sectional design. All data were collected from the 101 subjects during a 60 minute outpatient clinical research clinic visit for which subjects fasted overnight, and withheld morning medications, tobacco, caffeine and vigorous exercise the day of their study visit (Lee, et al., 2012). The protocol included history taking, obtaining physiologic measures, followed by blood and urine sample collection that was performed at a mean 62 days ± 33.8 days post catheterization (95% confidence interval (CI) [55.1, 68.4]). Full procedures including the medications that were withheld and for how long are described in Chapter 3 and are published elsewhere (Lee, et al., 2012). Significant exclusion criteria were put in place to avoid confounding the measures of inflammation and blood pressure. These exclusions consisted of

refraining from tobacco, caffeine, and vigorous exercise the morning of the study visit, and refraining for seven days prior to the visit from Vitamin C and E, fish oil, niacin, decongestants, erectile dysfunction drugs and anti-inflammatories other than low-dose aspirin (Lee et al., 2012).

Preliminary Examination of Data

Secondary data for all research questions were analyzed using Statistical Package for the Social Sciences (SPSS), Version 20 (International Business Machines Corp, 2012). There were no paper instruments utilized within this study, thus data analysis was able to begin immediately upon approval by the UNC, Greensboro Institutional Review Board and analyses of the serum to determine Vitamin D levels and hepatic function testing. The initial data analyses involved checks and re-checks for accuracy. The Principal Investigator (PI), when entering the laboratory values into the SPSS database, checked and re-checked the serum Vitamin D and hepatic panels (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]) analyses by double entry assessing for an exact match, and remedied any discrepancies against the original laboratory reports from Laboratory Corporation of America (Labcorp) (Burlington, North Carolina). Because these Vitamin D levels and hepatic analyses were not added to the medical records of the participants and were conducted for research purposes only, Labcorp did not perform customary quality control testing as is a standard for diagnostic decisions.

There were no missing data for these analyses. Much of the data were interval level, except for some binary or nominal level data such as sex, race, the diagnosis of HTN and depression for which frequencies were calculated. Nominal level data were recoded numerically for further statistical analyses. Descriptive statistics were calculated for all interval and ratio level data with mean, standard deviation (*SD*), standard error, kurtosis, skewness and 95% confidence intervals were reported. The PI conducted a search for outliers by observing boxplots, and assessing for points of influence or outliers, which often do not follow a normal distribution,

by examining DFBetas and Cook's Distance. Because outliers skew correlations and means and can also contribute to regression findings, calculations were performed with and without outliers. Additionally, combinations of variables within regression analyses were assessed by scatterplot to check for linearity of the relationships, for autocorrelation by the Durbin Watson statistic, for multicollinearity by variance inflation factors (VIFs) and tolerance factors, and for normality of residuals by boxplots and Kolmogorov-Smirnov (K-S) tests of the studentized deleted residuals (SDRs) for linear regression and the standardized residuals for logistic regressions.

Sample Demographics

Males comprised two-thirds (68 of 101) of the total sample. Mean age was 58.5 years (*SD* ± 9.3), and 81% were White (see Table 3). The proportion of subjects with a current diagnosis of hypertension (HTN) was 81%. In the full sample, 24% were diabetic, 38% had experienced a prior heart attack, 57% had had a prior positive cardiac catheterization, and a few (3%) reported a prior stroke. A diagnosis of depression was validated in 27% of the medical records. Subjects were receiving state-of-the-art cardiac care at a tertiary care hospital, and this exemplary care included 97% being on aspirin therapy, 93% on lipid lowering drugs (HMG-CoA [5-hydroxy-3-methylglutaryl-coenzyme A reductase] or statin drug), 82% on a beta blocker, 80% on Clopidogrel, 60% on an angiotensin-converting enzyme inhibitor (ACE), and 5% on an angiotensin receptor blocker (ARB). Few (23%) currently smoked, and of these smokers, only 13% (n = 3) reported smoking the morning of the study despite instructions not to do so. Mean body mass index (BMI) was in the obese range (30.4 ± 5.65) according to the Centers for Disease Control and Prevention (CDC) definition (CDC, 2011). A normal BMI (18.5-25) was present in 20% of subjects, while 26% were overweight (BMI 25-29.9), and 54% obese (BMI ≥ 30).

Table 3

Sample Demographic Statistics and Frequencies (N=101)

Background Variables	*N* or Mean ± *SD*	%*
Sex, male	68	67
Race		
White	82	81
Black	19	19
Age	58.5 ± 9.3 (Range 35-78)	
BMI, overall	30.4 ± 5.6 (Range 17-43)	
BMI, normal	20	20
BMI, overweight	26	26
BMI, obese	54	54
Prior positive cardiac catheterization	63	62
Prior myocardial infarction	58	57
Smokers	23	23
Hypertension diagnosis	82	81
Depressed	74	73
Non-depressed	27	27
Medications taken		
Aspirin	98	97
Plavix	81	80
HMG co-reductase (statin)	94	93
Beta blocker	83	82
ACE	56	56
ARB	5	5

Note. *SD* = standard deviation. *All percentages rounded up at 0.5%. HMG = 5-hydroxy-3 methylglutaryl coenzyme A reductase. ACE = angiotensin converting enzyme inhibitor. ARB = angiotensin receptor blocker.

The Puglisi Model of Vitamin D Levels' Associations with Depression

The Puglisi model involves both direct and indirect effects of Vitamin D levels upon depression and is depicted in Figure 1. Descriptive statistics with means or percentages are provided in Table 4 for the variables within the Puglisi model which guided this study.

Preliminary Data Examination and Variable Descriptive Statistics

Descriptive statistics were calculated for all non-demographic continuous variables to include their mean, *SD*, standard error, kurtosis, skewness and normality assessment by K-S tests. These descriptive statistics are outlined in Tables 3 and 4. Due to the substantial skewness for systolic blood pressure (SBP), diastolic blood pressure (DBP), high sensitivity C-reactive protein (hs-CRP), reactive hyperemia index (RHI) and augmentation index (AI), these variables were log transformed. A log transformation of AI was accomplished by adding a constant of 17 to all values because the lowest AI value was a negative number (-16). Unfortunately, the resulting skewness and kurtosis from this Log-AI were greater than the original AI skewness and kurtosis (log-AI skewness, -2.44 versus AI skewness, 0.95; log-AI kurtosis, 10.90 versus AI kurtosis, 0.63) which is the reason that the Log-AI was not used in any further analyses.

Independent measures and normality are important for *t* tests and regressions (Mertler & Vannatta, 2013). Univariate analyses to examine for outliers (by boxplots) are required for *t* tests, as well as for regression, which also require normality of residuals. Normality assessment by the K-S test is also included in Table 4, and these results indicated that hs-CRP was the only non-normal variable ($p \leq 0.001$). Consequently, the log transformation of hs-CRP (Log-hsCRP) was used for further analyses because it was robustly normally distributed per a K-S test ($p = 0.62$). Using a one-sample K-S test, AI and its log transformation (log) were normally distributed (*p* value for AI = 0.057; *p* value for log-AI = 0.06). Thus, the PI chose to use AI in the further

analyses due to the aforementioned smaller skew and kurtosis and similar normality values by K-S testing.

Specific Aim 1—Research Question 1

What proportion of the sample is depressed?

The proportion was calculated as a frequency and percentage for those depressed. Within the sample of CAD subjects (N = 101), 27 (or 27%) were depressed, (95% CI [19.1, 36.1%]).

Specific Aim 1—Research Question 2

What are the mean Vitamin D levels, serum hs-CRP level, BAFMD, RHI, and AI in those who are and are not depressed?

Descriptive statistics were calculated for the above continuous variables to include their mean, *SD* and range by depression group. These values are listed in Table 4, and differ from the overall sample means for which the Vitamin D levels, hs-CRP and RHI have lower values in the depressed, and BAFMD and AI have higher values in the depressed group.

Table 4

Descriptive Statistics for Continuous Variables (N = 101)

Variable	Mean	Standard error of Mean	Standard Deviation	Range	Skewness	Kurtosis	K-S normality *p* value
SBP	136	1.66	16.62	104 – 192	0.93	1.30	0.426
Log SBP	4.91	0.01	0.12	0.6 – 4.6	0.53	0.53	0.828
DBP	80	1.03	10.38	62 – 120	1.27	2.84	0.249
Log DBP	4.37	0.01	0.12	0.66 – 4.13	0.76	1.21	0.617
hsCRP	3.01	0.35	3.53	0.2 – 15.1	2.00	3.48	0.000
Log hsCRP	0.54	0.11	1.07	-1.6 – 2.7	0.20	-0.73	0.624
BAFMD	5.04	0.34	3.45	-1.0 – 14.0	0.46	-0.44	0.542
RHI	2.19	0.07	0.71	1.0 – 5.0	0.95	0.52	0.059
Log RHI	1.51	0.03	0.31	0.2 – 1.5	0.38	-0.71	0.418
AI	23.49	2.12	21.26	-16.0 – 95.0	0.95	0.63	0.057
Log AI	3.53	0.07	0.71	-0.3 – 4.7	-2.44	10.89	0.064
Vitamin D	21.05	0.24	9.15	5 – 41.4	0.56	- 0.02	0.036
Log Vitamin D	2.94	0.05	0.47	1.6 – 3.8	- 0.50	- 0.08	0.033

Note: hs-CRP = high sensitivity C-reactive protein; BAFMD = brachial artery flow mediated dilation; RHI = reactive hyperemia index; AI = augmentation index. Decimals rounded at 0.5%.

Table 5

Means of Continuous Variables by Depression Group (N = 101)

Variable	Mean ± *SD* or *N* (%)			Range	
	N = 101 Total sample	*n* = 27 Depressed	*n* = 74 Non-depressed	*n* = 27 Depressed	*n* = 74 Non-depressed
Vitamin D	21.05 ± 9.15	18.96 ± 7.02	21.81 ± 9.74	5.00 – 30.80	5.30 – 46.40
hs-CRP	3.01 ± 3.53	2.81 ± 3.26	3.08 ± 3.64	0.50 – 13.10	0.20 – 15.10
BAFMD	5.04 ± 3.45	5.62 ± 3.31	4.82 ± 3.50	1– 14	-1 – 13
RHI	2.19 ± 0.71	2.01 ± 0.77	2.25 ± 0.67	1 – 5	1 – 4
AI	23.49 ± 21.26	25.57 ± 24.40	22.74 ± 20.11	0 – 95	- 16 – 71

Note: hs-CRP = high sensitivity C-reactive protein; BAFMD = brachial artery flow mediated dilation; RHI = reactive hyperemia index; AI = augmentation index. Decimals rounded at 0.5%.

Specific Aim 1—Research Question 3

What proportion (%) of the sample has systolic and diastolic HTN?

The proportion of HTN was calculated to reflect those with a medical diagnosis of HTN as noted within participants' medical records, and not determined by the standard definition of HTN which is either a SBP ≥ 140 or DBP ≥ 90 mm Hg (The Joint National Committee on Prevention, 2004). Of the sample's 101 subjects, 82 or 81% had a current diagnosis of HTN (95% CI [72.5, 87.6]). Subjects had been instructed to hold their morning medications, and despite this sample being an aggressively treated cohort wherein one might expect blood pressures to still be reasonably well controlled when a dose of medication was only a few hours late, 11 of 101 or 11% of subjects had both elevated SBP and DBP, and 37 of 101 or 37% had an elevation of either SBP or DBP.

Specific Aim 1—Research Question 4

Is there a difference in Vitamin D levels, SBP, DBP, HTN, hs-CRP, BAFMD, RHI, and AI in those who are and are not depressed?

Basic means, *SD*, and frequencies were calculated for the variables in this question (see Table 4). To answer 1-RQ4, three statistical tests were used: (a) *t* tests, (b) Mann Whitney *U* tests for the variables with outliers, and, (c) a Chi-square for the HTN by depression question. Because BAFMD, AI, RHI, log-hsCRP, SBP and DBP were all normally distributed by K-S tests (Table 4), initial assumptions of *t* tests were met**.** Neither hs-CRP ($p \leq 0.001$) nor Vitamin D levels ($p = 0.036$) were normally distributed, but log-hsCRP was normally distributed ($p = 0.624$). Thus, log-hsCRP was utilized in further analyses because the homoscedasticity and normality assumptions were met. Because the log transformation of Vitamin D levels was also not normally distributed ($p = 0.033$), Vitamin D levels were utilized for further analyses in a Mann Whitney *U* test.

The last assumption of *t* tests is that there must be no extreme outliers. Only BAFMD and the log-transformed hs-CRP (Log-hsCRP) lacked boxplot outliers which allowed for *t* tests. There were, however, outliers which precluded the use of *t* tests for: (a) RHI (one outlier), (b) AI (two outliers), (c) Vitamin D levels (two outliers), (d) SBP (three outliers) and, € DBP (four outliers). Log trans-formations of AI, SBP, DBP and Vitamin D also resulted in outliers. The Log-RHI did not have outliers and was normally distributed by a K-S test ($p = 0.418$), so Log-RHI was utilized in a *t* test because RHI had boxplot outliers. For the variables with outliers or lack of normality (AI, Vitamin D, SBP and DBP), the Mann Whitney *U* test was chosen because the assumptions of having at least an ordinal dependent variable (depression or not) and independent observations were assured in a test that allows a comparison of means between groups. Thus, *t* tests were run for only BAFMD, Log-hsCRP and Log-RHI, while the non-

parametric Mann-Whitney *U* test was utilized for those Ivs (Vitamin D, SBP, DBP and AI) with outliers or lack of normality to assess differences in depression prevalence.

Table 6

Description of Variables Potentially Associated with Depression (N = 101)

Variable	N or Mean ± *SD*	*N* (%)
Vitamin D		
Mean	21.05 ± 9.15	
Normal (≥30ng/mL)	18	18
Insufficient (20-29.9 ng/mL)	31	31
Deficient (≤ 20 ng/mL)	51	52
Hypertension diagnosis (HTN)	82	81
No hypertension diagnosis	19	19
Vascular Events		
Prior myocardial infarction	38	38
Prior cerebrovascular accident	3	3
Current Smoker	23	23
Non-smoker	78	77
Smoked morning of Research Visit	3	3
Systolic Blood Pressure	136 (104-192 mm Hg)	
Diastolic Blood Pressure	80 (62-120 mm Hg)	
Endothelial Dysfunction Markers		
hs-CRP	3.01 (0.2 – 14.9 mg/L)	
BAFMD	5.04 (-1 – 14)	
RHI	2.19 (1 – 5)	
AI	23.49 (-16 – 111)	

Note: *percentages rounded at 0.5%. hs-CRP = high sensitivity C-reactive protein; BAFMD = Brachial artery flow-mediated dilation; RHI = reactive hyperemia index; and, AI = augmentation index.

The *t* tests indicated no significant difference by depression group for BAFMD (t = -1.022, df = 99, p = 0.269) and Log-hsCRP (t = - 0.25, df = 99, p = 0.80), but a difference for Log-RHI (t = 1.97, df = 99, p = 0.05) was noted. Assumptions were met for the Mann Whitney U test, and there were no differences by depression group for Vitamin D levels (p = 0.25), DBP (p = 0.33), SBP (p = 0.97), nor for AI (p = 0.99) (see Table 7).

The final analysis for this question was to examine the differences in HTN by depression group. This was conducted by a Chi-Square test. The Chi-Square assumption of expected frequencies of 5 or more per cell was met (Huck, 2008). Twenty-seven of the 101 CAD patients were depressed, with 23 (85%) having HTN and only four of those with depression had no HTN diagnosis. The relationship between having a diagnosis of HTN and depression, however, was not significant by Chi-Square (X^2 = 0.39 (2, 99) = 0.39, p = 0.54) (see Table 7).

Table 7

Differences in Vitamin D Level, Measures of Blood Pressure and Inflammation by Depression Group

Variable	Mean ± *SD* or *N (%)*		*p* value
	Depressed *n* = 27	Non-depressed *n* = 74	
Vitamin D	18.96	21.81	0.25 [a]
Systolic Blood Pressure	134.25	136.99	0.97 [a]
Diastolic Blood Pressure	77.86	80.33	0.33 [a]
hs-CRP*	0.58	0.52	0.80 [b]
Reactive Hyperemia Index*	0.64	0.77	0.05 [b**]
Augmentation Index	24.69	23.03	0.99 [a]
BAFMD	5.42	4.89	0.31 [b]
Hypertension	27 (27%)	74 (73%)	0.54 [c]

Note: BAFMD = brachial artery flow mediated dilation. * = log transformed variables. *P* values obtained by different tests and rounded at 0.5. [a] Mann-Whitney *U* tests; [b] t tests; [c] Chi-square test for discontinuous variables. ** $p = 0.052$.

Specific Aim 2—Research Question 1

Is Vitamin D level associated with depression?

The state of having depression was noted in the medical record and was binary (depressed = 1, non-depressed = 0). Assumptions were met for logistic regression, including: (a) the dependent variable (DV) was dichotomous (depression), (b) measures were independent within this sample, (c) there were no extraneous variables included, (d) there was no multicollinearity, and € the IV was assumed to be measured without error (Mertler & Vannatta, 2013). There were no outliers by sample-size adjusted DfBetas > 0.20 ($2\sqrt{101}$)for a value more

than 0.20 for Vitamin D level (Tabachnick & Fidell, 2007), and only two non-extreme outliers in the boxplots of Vitamin D levels, so the regression was run with and without these outliers. Further, tertiles of Vitamin D sufficiency (normal > 30 ng/mL, insufficient 20-29.9; ng/mL, and deficient < 20 ng/mL) were used as independent variables in a second logistic regression.

The logistic regression of the whole dataset (N = 101) to explore continuous Vitamin D levels association with a diagnosis of depression yielded a statistically significant Hosmer and Lemeshow test (p = 0.029) which indicates a lack of fit to the model. This overall model was not significant (AOR 0.97; p = 0.17; 95% CI [.92, 1.02]) (see Table 8). The logistic regression of data minus the two outliers for Vitamin D levels (N = 99) revealed a good fit by the Hosmer and Lemeshow test (p = 0.078), but Vitamin D level was still not significantly associated with depression (AOR 0.97; p = 0.26; 95% CI [.92, 1.02]). A final logistic regression was performed using tertiles of Vitamin D sufficiency levels to determine their association with depression. Results indicated a statistically reliable model (Hosmer and Lemeshow p = 0.17), but as with the continuous Vitamin D level models, Vitamin D levels were not significantly associated with depression by tertiles: (a) for deficient versus normal Vitamin D level (AOR = 0.31; p = 0.15; 95% CI [.6 , 1.51]), nor for (b) insufficient versus normal Vitamin D level (AOR = 1.18; p = 0.74; 95% CI [.45, 3.08]).

Table 8

Logistic Regression Coefficients for Vitamin D's Association with Depression (N = 101)

	b	Exp(B)	95% Confidence Interval Exp(B)	*p* value
Vitamin D	-0.037	0.96	0.93 – 1.02	0.17

Specific Aim 2—Research Question 2

When controlling for age, sex, race and BMI, do Vitamin D levels differ in those with and without depression?

A logistic regression was performed to examine the association of Vitamin D levels with depression. Assumptions for a logistic regression were met as previously described. Bivariate correlations (see Table 9) demonstrated no correlations above the acceptable limit of 0.80 between variables (Mertler & Vannatta, 2013). Assessing for outliers by a sample size adjusted DFBetas ($2\sqrt{101}$) for a value more than 0.20, and by Cook's Distance being set at 4/(n – k – 1) which in this case is 4/(101 – 5 – 1) resulted in a Cook's cutoff of 0.042 (Tabachnick & Fidell, 2007). There were no sex DFBeta outliers. Age and race had five outliers, Vitamin D levels eight outliers, and BMI 14 outliers. Additionally, an examination of Cook's Distance points revealed 6 unique cases. Thus, there were 22 total unique outliers when adding the cases of influence for Vitamin D levels, BMI and race. Thus, the decision was made to run this analysis with (N = 101) and without (N = 79) these outliers.

Table 9

Correlations of Continuous Variables with Associations Reported in Prior Published Literature

Variables	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1. Age	1.00	-.15	.19	-.31˜	-.29˜	-.09	-.32˜	-.34˜	.13	-.02	.07	.11
2. BMI		1.00	-.15	-.02	-.17	-.08	.16	.10	-.11	-.21*	.29˜	-.05
3. SBP			1.00	.56˜	.23*	.04	-.11	-.16	.16	.37˜	.02	-.32˜
4. DBP				1.00	.25*	.05	.08	.15	.07	.23*	.14	-.25
5. eGFR					1.00	.01	.12	-.07	.12	.23*	-.06	-.16
6. AST						1.00	.74˜	-.05	-.001	.09	-.10	.15
7. ALT							1.00	.11	-.05	-.07	-.12	.17
8. BAFMD								1.00	-.08	.04	-.04	-.09
9. RHI									1.00	.31˜	.05	-.08
10. AI										1.00	-.08	-.30˜
11. hsCRP											1.00	-.20*
12. Vitamin D												1.00

Note: BMI = body mass index; eGFR = estimated glomerular filtration rate; AST = aspartate aminotransferase; ALT = alanine aminotransferase (ALT); BAFMD = brachial artery flow mediated dilation; . RHI = reactive hyperemia index; AI = augmentation index; and hs-CRP = high sensitivity C-reactive protein.

***** $p \leq 0.05$

˜ $p \leq 0.01$

The demographic variables (age, sex, race and BMI) were entered into the first block and Vitamin D levels in the second block. The final model yielded a reliable model (Hosmer and Lemeshow, $p = 0.65$). However, the overall model did not depict a significant association between the demographic variables, Vitamin D and the diagnosis of depression (AOR 0.96; $p = 0.13$; 95% CI [.90, 1.01]) (see Table 10). The model without the two Vitamin D outliers had a

reliable model (Hosmer and Lemeshow, $p = 0.94$), but it also did not depict a significant association between the demographic variables, Vitamin D levels and the diagnosis of depression (AOR 0.96; $p = 0.22$; 95% CI [.91, 1.02]). Next, the analyses was conducted without the 22 outliers ($N = 79$). This model significantly fit the data (Hosmer and Lemeshow $p = 0.61$), but again, the demographic variables and Vitamin D levels were not significantly associated with depression (AOR 0.96; $p = 0.14$; 95% CI [.80, 1.03]) (see Table 10).

Table 10

Regression Coefficients for the Association of Vitamin D Levels, Demographic Variables and Depression (N = 101)

	b	Exp(B)	95% Confidence Interval Exp(B)	*p* value
Age	-0.16	0.984	0.93 – 1.037	0.55
Sex	-0.99	0.37	0.14 – 0.98	0.05
Race	-1.36	0.26	0.06 – 1.07	0.06
BMI	0.03	1.03	0.95 – 1.17	0.48
Vitamin D level	-0.05	0.96	0.90 – 1.01	0.13

Note: BMI = body mass index.

Specific Aim 3—Research Question 1

What is the relationship of Vitamin D levels to measures of HTN (SBP, DBP, yes/no HTN)?

Logistic regression was employed to model the relationship of Vitamin D levels to a medical diagnosis of HTN. For the logistic regression, assumptions were previously assured.

Then, a multivariate multiple regression (MR) analysis of the association of Vitamin D levels with SBP and DBP was conducted.

Hypertension was coded as a dichotomous variable and a logistic regression was used to determine the association with Vitamin D levels with HTN. The full model (N = 101) revealed a good fit (Hosmer and Lemeshow, p = 0.53), but there was not a significant association between Vitamin D levels and HTN (AOR 0.97; p = 0.28; 95% CI [.92, 1.02]). All cases of HTN were correctly predicted in this model, but none of those without HTN were predicted.

A multivariate MR was used to explore Vitamin D levels' associations with SBP and DBP. Multicollinearity can occur in situations of bivariate analyses, such as between Vitamin D levels and HTN (yes/no), so it was important to examine the Spearman's rank correlation of HTN and Vitamin D levels which was found to be $r = -0.10$ (p = 0.37), thus, indicating no multicollinearity issues. Additionally, the Pearson's correlations of SBP and Vitamin D levels (r = - 0.32, p = 0.001) and DBP and Vitamin D levels (r = - 0.25, p = 0.01) were significant but not greater than the acceptable limit of 0.80 indicating the variables were appropriate to utilize in linear regression models (Mertler & Vannatta, 2013).

Prior to performing the multivariate MR with SBP and DBP, a search was conducted for potential violations that might affect this multivariate regression. Data were inspected for outliers, linearity and multicollinearity issues. There was no multicollinearity between Vitamin D levels and SBP or DBP because their VIFs were less than 1.5, and tolerance factors all > 0.10 (Mertler & Vannatta, 2013. Normality of the studentized deleted residuals was noted by K-S tests for both SBP (p = 0.65) and DBP (p = 0.29). Linearity was approximated between Vitamin D and both SBP and DBP. There was no autocorrelation between Vitamin D levels and SBP (Durbin Watson = 1.87) or for DBP (Durbin Watson = 1.84) (Norusis, 2008). Boxplots showed outliers for SBP (three cases); DBP (four cases); and Vitamin D level (two cases). Because

multivariate and linear regressions require assumptions to be met including normality, linearity *and* no significant outliers, the regression was run a second time without these 7 outlier points from the blood pressures ($N = 94$). Further analyses for significant points of influence were performed by examination of DFBeta and Cook's Distance. The DFBeta cut-off point was again sample size adjusted as previously described and was 0.20, and a Cook's Distance cut-off point of 0.04 reflected a point of influence. A total of 10 unique points found by the analysis of both DFBeta and Cook's Distance were removed and an additional regression ($N = 91$) conducted.

Prior to performing the multivariate MR of the association of Vitamin D levels and blood pressures, consideration was given to other correlations that would be expected from the literature. The associated Pearson *r* values when statistically significant are listed for each association in Table 9. Other expected relationships from the literature include: (a) older age and increased log-hsCRP, (b) lower Vitamin D levels associated with older ages (Holick, 2007), (c) older age and increased prevalence of CAD (CDC, 2012 (d) older age and increased AI (Janner, et al., 2012), (e) less prevalence of depression in those over age 60 (although 31% were depressed in this sample of those > 60 years and 77% with depression in those < age 60) (Kessler, et al., 2005), (f) increased diagnoses of HTN in older ages, with 52% having HTN at ages 55-64 (Writing Group for the American Heart Association, 2013), (g) lower Vitamin D levels in those with reduced estimated glomerular filtration rate ($r = -0.16$, $p = 0.11$) (eGFR) and reduced liver function by ALT ($r = 0.19$, $p = 0.06$) and AST ($r = 0.18$, $p = 0.08$) (Putz-Bankuti, 2012), and (h) increased blood pressures and decreased endothelial function (hs-CRP, BAFMD, RHI, PAT) in those with CAD [r of DBP and AI = 0.23, $p = 0.02$] (Jablonski, et al., 2010). All correlations were less than the acceptable limit of 0.80 indicating the variables were appropriate to utilize in linear regression models (Mertler & Vannatta, 2013) (see Table 9).

The multivariate multiple regression was run on the full sample (N = 101) to model the association of Vitamin D with SBP and DBP. The overall multivariate regression for Vitamin D on SBP and DBP was significant (p = .004). Regression results indicate that Vitamin D was significantly associated with SBP (R^2 = 0.10; R^2_{adj} = 0.09; df 1; F = 10.88; p = 0.001) and DBP (R^2 = 0.06; R^2_{adj} = 0.05; df 1; F = 6.64; p = 0.011). Another multivariate regression for Vitamin D on SBP and DBP was then run excluding the 10 outliers (N = 91). This model was also significant for SBP (R^2 = 0.15; R^2_{adj} = 0.14; df 1; F = 15.69; $p < 0.001$) and for DBP (R^2 = 0.10; R^2_{adj} = 0.09; df 1; F = 9.49; p = 0.003). Significant results in the multivariate model indicated that further analyses by simple linear regression for the individual variables (SBP and DBP) were appropriate.

Simple linear regressions were next run to model the effect of Vitamin D levels upon SBP and DBP (see Table 11). Assumptions were checked as previously described including normality of the studentized deleted residuals for both SBP (p = 0.65) and DBP (p = 0.29) by K-S tests. Both simple linear regression models for SBP and DBP were again significant. Vitamin D level was significantly associated with SBP (B = - 0.57, 95% CI [- .92, – .23]; F = 10.88 (*1*, 99); p = 0.001) and for DBP (B = - 0.28; 95% CI [–.50 , -.07]; F = 6.64 (*1*, 99); p = 0.011). Thus, for every increase in 1 ng/mL of Vitamin D, the odds were that DBP decreased 0.28 mm Hg (p = 0.011) and SBP decreased 0.57 mm Hg (p = 0.001).

Table 11

Regression Coefficients for the Association of Vitamin D and Blood Pressures (N = 101)

	Unstandardized Coefficient β	95% CI for Coefficient β	*p* value	R^2
DBP	- 0.28	- 0.50 to – 0.07	0.011	0.063
SBP	- 0.57	- 0.92 to – 0.23	0.001	0.099

Note: DBP = diastolic blood pressure; SBP = systolic blood pressure; decimals rounded up at 0.5%

Specific Aim 3—Research Question 2

What is the relationship of Vitamin D levels to measures of inflammation (hs-CRP, BAFMD, RHI, and AI)?

To answer this question, log-hsCRP, BAFMD, RHI and AI were entered into a multivariate MR and two regressions were run: one with Vitamin D levels continuously as the IV and the other with Vitamin D levels in tertiles as the predictor variable to any association with inflammation. Assumptions were checked including: (a) linearity between IV and DV each (by scatterplot), (b) normality of the studentized deleted residuals, (c) independence of errors by the Durbin-Watson statistic which is typically between 1.5 to 2.5 when independence is present (Norusis, 2008), (d) homoscedasticity (by scatterplot of the studentized deleted residuals and/or a non-significant *p* value on a K-S test), (e) appropriate variables, or parsimony, within the model, (f) no multicollinearity between the IVs, and, (g) no extreme outliers present (Mertler & Vannatta, 2013). Because multiple regression is sensitive to extreme outliers, merely one or two outliers can affect results; therefore, dealing with the extreme cases by identifying and removing them is helpful (Mertler & Vannatta, 2013). Only one variable, hs-CRP, had extreme outliers by boxplot, hence, the Log-hsCRP was used in this multivariate model. There were several non-

extreme (less than 1.5 *SD* outside of the mean) in the boxplots: (a) RHI (one outlier), (b) AI (one outlier), (c) Vitamin D levels (two outliers), and (d) Log-Vitamin D level (two outliers). However, while regressions can be sensitive to one or two outliers (Mertler & Vannatta, 2013), one or two outliers are to be expected in a large data (T. P. McCoy, personal communication, September 30, 2013). Thus, these outliers were not felt to violate the assumptions of multivariate multiple linear regression.

Linearity was present between the IV and the multiple DVs, and there were no high correlations between the variables (see Table 9). The studentized deleted residuals demonstrated normality by non-significant K-S test results: RHI ($p = 0.06$), AI ($p = 0.40$), BAFMD ($p = 0.60$), and Log-hsCRP ($p = 0.84$). Scatterpolts of the predicted values and residuals were examined. The points were equally distributed around the midpoint, with no concerning patterns noted (Tabachnick & Fidell, 2007). No high correlations between variables and VIFs and tolerance factors were within accepted ranges indicated no multicollinearity. The multivariate MR model of Vitamin D levels association with measures of inflammation was significant ($p < 0.001$).

Because the multivariate model was significant for all four measures of inflammation, individual simple linear regressions (SLR) were performed to explore the relationship between each measure of inflammation and Vitamin D (see Table 12). Normality of residuals was also present in the simple linear regression residuals, and assumptions, as previously described, were met. The linear regression between Vitamin D and AI was significant indicating an association between Vitamin D level and AI ($R^2 = 0.09$; $R^2_{adj} = 0.08$; F (1, 99) = 9.67; $p = 0.002$). Results of the SLR associating Vitamin D levels with Log-hsCRP were also significant ($R^2 = 0.12$; $R^2_{adj} = 0.11$; F (1, 99) = 13.10; $p < 0.001$). The two other independent variables, BAFMD and RHI, were not significantly associated with Vitamin D levels.

Table 12

Regression Coefficients for the Association of Vitamin D Levels and Measures of Inflammation (N = 101)

Variable	b	95% CI for b	β	*p* value	R^2
Log-hsCRP	- 0.04	- 0.06, - 0.02	- 0.34	< 0.001	0.12
AI	- 0.69	- 1.14, - 0.25	- 0.30	0.002	0.09
BAFMD	- 0.32	- 0.39, - 0.11	- 0.86	0.394	0.007
RHI	- 0.01	- 0.02, - 0.01	- 0.84	0.401	0.007

Note: Log- hsCRP = log transformation of the high-sensitivity C-reactive protein; AI = augmentation index; BAFMD = brachial artery flow mediated dilation; RHI = reactive hyperemia index. All decimals rounded at 0.5%.

Specific Aim 3—Research Question 3

Are measures of HTN (SBP, DBP, yes/no HTN) and measures of inflammation (hs-CRP, BAFMD, RHI, and AI) associated with the occurrence of depression?

An exploratory logistic regression was performed utilizing forward stepwise regression with HTN measures entered in Block 1 and inflammation measures entered in Block 2. Assumptions for a logistic regression were met, including that the DV was dichotomous (depression), measures were independent within this sample, there were no known extraneous variables included, there were no extreme outliers, and the IV was measured without error (Mertler & Vannatta, 2013).

This forward stepwise logistic regression yielded a reliable model (Hosmer and Lemeshow, $p = 0.42$). Regression results revealed that the model predicted 100% of those without depression but none of those with depression, but the overall model was not significant ($p = 0.66$). Significantly concerning outliers for this sample of 101 were set as DFBeta values ≥

0.20, and Cook's Distance values ≥ 0.043 (adjusted for the sample size of 101 by 4/(n - k - 1) or 4/101 – 7 – 1). There were no concerning points by Cook's distance, and DFBetas were not calculated per variable because the first iteration took out all three HTN measures and the second iteration all four inflammation variables. Thus, this model was not significant ($p = 0.66$). As an additional analysis, the order of Block 1 and 2 were reversed with inflammation measures in Block 1 entered by forward Wald and HTN measures in Block 2 by forward Wald method. The results again revealed lack of significance ($p = 0.33$).

Specific Aim 3—Research Question 4

When controlling for age, sex, race and BMI, are Vitamin D levels, serum and endothelial measures of inflammation, and measures of HTN associated with depression?

This question was answered by logistic regression because depression is binary and utilized the forward stepping Wald method due to the exploratory nature of the question. Forward stepping regression assures that only IVs that predict the DV in a significant manner will be utilized within the final model. The resulting model was reliable (Hosmer & Lemeshow, $p = 0.65$). The model correctly predicted 95% of those without depression and 26% of those with depression. There were no missing cases, and the assumptions for the logistic regression were met. High correlations did not exist between the IVs as Table 9 demonstrated, and VIFs (all under 2.4) and tolerance factors (all above 0.10) validated a lack of multicollinearity. Extreme values of IVs were avoided by assessing the standardized residuals to detect outliers. DFBetas were examined for outliers of all 12 predictor variables for the sample size adjusted cut-off for a significant DFBeta point (0.20). Analysis of outliers by Cook's Distance revealed six outliers. Consequently, this question was answered with ($N = 101$) and without ($N = 95$) these six influential points.

The overall model for N = 101 subjects was reliable (Hosmer and Lemeshow p = 0.65), but not significant (p = 0.13). In fact, all blood pressure and inflammation measures were removed from the overall model. The final model is depicted in Table 13: AOR 0.96; p = 0.13; a 95% CI [.90, 1.01]. The logistic regression model without the 6 outliers also revealed a reliable model (Hosmer and Lemeshow, p = 0.80) but was also not significant (AOR 0.95; p = 0.17; 95% CI [.89, 1.02]).

Table 13

Regression Coefficients for the Association of Demographic Factors, Vitamin D Levels, and Measures of Inflammation and Hypertension upon Depression (N = 101)

Variable	b	Exp(B)	95% Confidence Interval Exp(B)	*p* value
Age	-0.16	0.984	0.934 – 1.037	0.55
Sex	-0.99	0.37	0.14 – 0.98	0.05
Race	-1.36	0.26	0.06 – 1.07	0.06
BMI	0.03	1.03	0.95 – 1.17	0.48
Vitamin D level	-0.05	0.96	0.90 – 1.01	0.13

Note: BMI = body mass index. Decimals rounded at 0.5.

Logistic regression is sensitive to the number of cases to variables within the analysis; if cases are too few, logistic regression can produce extremely large parameter estimates and standard errors. Sometimes researchers attempt to remedy this by collapsing variables or deleting offending categories of variables (Mertler & Vannatta, 2013). Even when rules are relaxed, it is typical to require at least 5-9 events per variable within a logistic regression (Vittinghoff & McCulloch, 2007). Thus with 27 subjects of 101 (27%), or in the model adjusted for outliers, 21

of 95 subjects (22%) with depression, utilizing a logistic regression with more than 5 or 6 IVs would not be optimal. For this reason, the effect of only HTN (yes/no), serum Vitamin D levels and Log-hsCRP was tested in the full model (N = 101), as well as in the model adjusted for outliers (N = 95) (see Table 14). Both overall reduced variable models were entered with demographic variables in Block 1, HTN in Block 2 by the Wald method, Log-hsCRP in Block 3 by the Wald method, and Vitamin D in Block 4. The overall (N = 101) reduced variable model was reliable (Hosmer and Lemeshow, p = 0.36) and was also not significant (AOR 0.96; p = 0.36; 95% CI [.90, 1.02]) (see Table 14). The reduced variable model adjusted for outliers (N = 95) was also reliable (Hosmer and Lemeshow p = 0.65) but was again not significant (AOR 0.96; p = 0.21; 95% CI [.90, 1.02]).

Table 14

Logistic Regression Coefficients for the Association of Demographic Factors, Hypertension, hs-CRP and Vitamin D Levels upon Depression (N = 101)

Variable	b	Exp(B)	95% Confidence Interval Exp(B)	*p* value
Age	-0.03	0.97	0.92 – 1.03	0.31
Sex	-1.05	0.36	0.13 – 0.94	0.04
Race	-1.56	0.21	0.05 – 0.91	0.04
BMI	0.24	1.03	0.95 – 1.11	0.56
Hypertension	0.85	2.34	0.60 – 9.19	0.22
Vitamin D level	-0.42	0.96	0.90 – 1.02	0.16

Note: BMI = body mass index. Decimals rounded at 0.5.

Specific Aim 3—Research Question 5

When controlling for age, sex, race, BMI and Vitamin D levels, are serum and endothelial measures of inflammation and measures of HTN associated with depression?

This question was again answered by logistic regression. Additionally, as noted in 2-RQ1, there was no significant association between Vitamin D levels (either as continuous or tertile variables) and depression, but the PI elected to add continuous Vitamin D levels as a variable into this final model. The enter method was used for the demographic variables and Vitamin D, but because this is an exploratory question, the forward stepping Wald method was used with the blood pressure measures in Block 2 (HTN, SBP, DBP), and the inflammation measures (BAFMD, AI, RHI and Log-hsCRP) in Block 3. Assumptions were assured as previously described for logistic regression. Significant outliers per Cook's Distance for the 12 IVs in this question would be 4/(101 - 12 IVs - 1) or 0.045. The assessment of the analogue of Cook's Distance revealed 6 outliers that presented themselves in analyses, and these points were all found within the influential DFBeta points. Influential DFBeta points > 0.20 were removed as follows: (a) five in age, (b) none in sex, (c) five in race, (d) eight in Vitamin D level, and (e) 14 in BMI. Thus, between the significant Cook's Distance points and the DFBeta points of influence, the sample size was adjusted downwards to $N = 78$.

Special attention was given to the possibility of multicollinearity between Vitamin D levels and blood pressure measures, and Vitamin D levels and measures of inflammation because these were found to be related in prior analyses. The overall model with all 12 IVs yielded a reliable model (Hosmer and Lemeshow, $p = 0.65$), but not a significant model (AOR 0.956; $p = 0.13$; 95% CI [.90, 1.01]). Even with relaxed rules, typically five to nine occurrences of the DV are required per IV in a logistic regression (Vittinghoff & McCulloch, 2007). Thus, because only 27 subjects of 101 had depression herein, an additional analysis was performed utilizing HTN to

represent a blood pressure variable and Log-hsCRP to represent an inflammation variable to keep the independent variables down to seven. This overall reduced variable model yielded a reliable result again (Hosmer and Lemeshow, $p = 0.65$) and yielded exactly the same result as the above full model (AOR 0.956; $p = 0.13$; 95% CI [.90, 1.01]). With the reduced sample that excluded the outliers ($N = 78$), the regression yielded a reliable model (Hosmer and Lemeshow, $p = 0.61$), but again was not significant (AOR 0.91; $p = 0.14$; 95% CI [.80, 1.03]).

Summary

The purpose of this study was to examine the association of serum Vitamin D levels, blood pressure measures, and inflammatory markers upon the prevalence of depression in a cross-sectional study of persons ($N = 101$) with CAD. Study measures included blood pressure measures, serum analysis of hs-CRP and non-invasive endothelial measurements obtained by an EndoPAT (Itamar Medical) and an ultrasonographer (for BAFMD). The sample was primarily White (81%), male (67%), had a mean age of 58 (± 9.3 years), with 38% having had a prior myocardial infarction, and 57% a prior positive catheterization. Most had HTN (81%) or diabetes (24%). The vast majority of Vitamin D levels (82%) were below normal (< 30 ng/mL). When depression was present (27%), it was not significantly associated with Vitamin D levels, even when controlling for demographic factors. However, Vitamin D levels were inversely associated with SBP ($p < 0.001$) and DBP ($p < 0.011$), although they were not associated with HTN) ($p = 0.28$). Lower Vitamin D levels were associated with increased inflammation. Hence, Vitamin D levels did not appear to significantly influence depression in this sample of persons with CAD, but there was an association between Vitamin D levels and blood pressures and some measures of inflammation.

CHAPTER V

DISCUSSION

Introduction

This study aimed to explain the differences in Vitamin D levels, hypertension (HTN), serum and endothelial measures of inflammation, and depression in persons with coronary artery disease (CAD). The relationships between serum Vitamin D levels and depression, blood pressure measures (HTN yes/no, systolic and diastolic blood pressures) and measures of inflammation, including high sensitivity C-reactive protein (hs-CRP), brachial artery flow mediated dilation (BAFMD), reactive hyperemia index (RHI), and augmentation index (AI) were explored. This chapter details the findings and the suggestions for implications for care by advanced practice nurses and other healthcare providers caring for persons with CAD. Future directions for additional research are also reviewed.

Demographics

Age and Sex

Subject mean age was 58.5 ± 9.3 years, and the sample consisted primarily of males (66%), which is typical for persons with CAD. This was an aggressively treated cohort who was receiving state of the art care at a major medical center in the Triad of North Carolina (NC). The sexes were closely matched in age: females mean 58.5 years ± 7.8 years with a 30 year range and males mean 58.5 years ± 10 years with a range of 43 years. In NC, 51% of the population is female and 14% of the population is over age 65 years (United States Census Bureau, 2012a). The location of the study, Orange County, NC, is home to a major university. Thus, it is not surprising that only 10.3% of Orange County residents are above age 65, compared to a state-

wide rate of 13.8% and the national rate of 14% above age 65 years (United States Census Bureau, 2012a; United States Census Bureau, 2012b). As expected for a study of persons with CAD, a large portion (22%) of this sample were above age 65 (United States Census Bureau, 2012b). This discrepancy in age prevalence rates between the county and the study sample is not surprising given that Orange County is home to a major university which likely draws more young people to the area, and that the prevalence of CAD increases with age (American Heart Association Statistics Committee and Stroke Statistics Committee et al., 2012).

Race

The majority of this central NC sample was White (81%), and the rest were Black. According to the United States Census Bureau in 2012, NC's population was 22% Black and 72% White (United States Census Bureau, 2012a). Therefore, this sample closely represents the proportion of Whites and Blacks within the state.

Body Mass Index

Within this sample, mean body mass index (BMI) was in the obese range (30.4 ± 5.65). According to the Centers for Disease Control and Prevention (CDC), the definition of obesity is a BMI of more than 30 kg/m^2 (CDC, 2011a). Subjects with a normal (BMI 18.5-25) constituted 20% of the sample, while 26% were overweight (BMI 25-29.9), and 54% obese ($BMI \geq 30$). This sample of persons with CAD appears different from the general sample that answered the NC Behavioral Risk Factor Surveillance System (BRFSS) because 54% of the sample as compared to 30% of those statewide, were obese. Some 70% of males and 60% of women in NC are overweight or obese which is much less than the 74% of males and 73% of females that were obese in another dataset (The Henry J. Kaiser Family Foundation, 2013).

It was not unexpected to find that this sample was more obese than the general population because of the sample's chronic disease states (CAD, HTN and diabetes), which likely leads to a

decreased functional capacity and perhaps even more edema, if the patient has some level of heart failure or anemia of chronic disease.

Renal and Hepatic Function

The sample's mean kidney function (estimated glomerular filtration rate, [eGFR]) was slightly below normal, but the mean hepatic functions, which were measured by aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were normal. Mean eGFR was 84.23 mLs/min (95% confidence interval (CI) [80.85, 87.60]; range 43-126 mLs/min) which is less than the 90 mLs/min that is considered normal (The Renal Association, n.d.). This is not surprising given that 82% of the sample had HTN which is a precursor to CAD (American College of Cardiology Foundation et al., 2012).

Sample mean AST was 21 IU/L (95% CI [20.8, 24.0]; range 10-59) and mean ALT was 25 (95% CI [22.9, 28.3]; range 6-90). Both of these sample means for AST and ALT fell within normal values for this lab (Laboratory Corporation of America [Labcorp]): AST normal = 0 – 40 IU/L and ALT normal = 0 – 32 IU/L. Because the liver is a key organ involved in the creation of metabolically active Vitamin D, it was theorized that hepatic function may affect Vitamin D levels (Jablonski et al., 2013; Putz-Bankuti et al., 2012).

Vitamin D Levels

Vitamin D levels when normal are above 30 ng/mL, are insufficient when between 20-29 ng/mL, and are deficient when < 20 ng/mL (Holick et al., 2011). In this sample, the overall mean Vitamin D level was insufficient (21.0 ng/mL ± 9.1, 95% CI [19.2, 22.9]). Vitamin D levels did not vary significantly by depression group (depressed mean 19.0, non-depressed 21.8, $p = 0.25$); however, Vitamin D levels did vary significantly by sex in this sample ($p \leq 0.001$). Men's Vitamin D levels were 19.3 (95% CI [16.2-22.3]) and women's were 21.9 (95% CI [19.6, 24.02]).

Vitamin D levels may have differed by sex because the small number of women in this sample had a much wider range of Vitamin D levels (male range 8.4 to 46.0; female's range 5.0 to 46.4).

Of this sample, 57% had a prior positive cardiac catheterization indicating established disease with perhaps a decreased functional capacity. Many CAD patients may have decreased exercise tolerance and thus, limited functional capacity to perform activities of daily living such as walking outside to a mailbox or spending leisure time in the sun to support adequate Vitamin D levels. Additionally, Vitamin D levels are difficult to maintain from diet alone because so few foods have large amounts which means that for most individuals, the major source of their Vitamin D level is from sun exposure which results in the conversion of 7-dehydrocholesterol (precursor Vitamin D) into active Vitamin D (Holick, 2007). Thus, it is not surprising due to perhaps a lesser ability to seek time outdoors that this sample was found to have an overall insufficient mean Vitamin D level (21 ng/mL; *SD* = 9.15; 95% CI [19.2, 22.86]), rather than a normal Vitamin D level (which is ≥ 30 ng/mL) (Holick et al., 2011). Thus, one would predict that there may be less sun exposure in a sample that consisted of primarily chronically ill persons because this study sample was comprised of 57% with CAD, 38% with a history of a prior myocardial infarction and 24% were diabetic.

Because the liver, and then the kidneys, are sites of hydroxylation that allow for synthesis of Vitamin D and because regression analyses require the assumption of no multicollinearity, the correlations were sought between Vitamin D levels, eGFR, AST and ALT. None of these Pearson *r* correlations were significant nor above the suggested cut-off of 0.80: (a) AST and Vitamin D levels $r = 0.18$, $p = 0.08$, (b) ALT and Vitamin D levels $r = 0.19$, $p = 0.06$, and (c) eGFR and Vitamin D levels $r = -0.16$, $p = 0.11$ (Allison, 2007). Only eGFR had an inverse relationship with Vitamin D level, but this was non-significant despite prior literature suggesting

a significant inverse relationship that has been proven in animals (Al-Badr & Martin, 2008; Vaidya & Williams, 2012).

Blood Pressure Measures

Hypertension

Hypertension is a well-established risk factor for CAD (American College of Cardiology Foundation, et al., 2012). Of the 101 persons with CAD in this dataset, 82 (81%) had a current diagnosis of HTN (95% CI [72.5%, 87.6%]), and this is consistent with 82% of hypertensive and 72% of normotensive patients undergoing coronary angiography having CAD (Zeina, Barmeir, Zaid, & Odeh, 2009).

The awareness, treatment and control of HTN is the highest in the Southeast United States (Olives, Myerson, Mokdad, Murray, & Lim, 2013). Prevalence of HTN increases with age and smoking; Black men and women have the highest total prevalence of HTN (Olives, Myerson, Mokdad, Murray, & Lim, 2013). This sample was comprised of only 19% Black persons despite the NC prevalence of 22% Blacks; this may have decreased the ability to find significance in the associations between HTN, SBP, DBP and race and may have decreased the odds of finding significance for other research questions.

Despite optimal cardiac care, 23% of this sample of persons with CAD smoked; in the United States in 2012, 21% of adult men and 17% of adult women smoked (American Heart Association Statistics Committee and American Stroke Statistics Committee, 2012). Consequently, it is not surprising that this data revealed a higher prevalence of tobacco use given that smoking is a known contributor to CAD. Significant findings may have occurred with the HTN, SBP and DBP variables due to the prevalence of 23% being smokers and/or the 13% (n = 3) of these smokers who reported smoking the morning of the study despite instructions not to do so.

Systolic and Diastolic Blood Pressures

The sample's blood pressure was well-controlled overall as would be expected at a major tertiary care center such as The University of North Carolina, Chapel Hill Hospitals. Mean SBP was 136.2 ± 16.6 and mean DBP was 79.6 ± 10.4 mm Hg which put these blood pressures within the normal range of SBP < 140 mm Hg and DBP < 90 mm Hg (The Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure [JNC], 2004). A few were hypertensive the morning of their study visit (35 with elevated SBP and 14 with elevated DBP) despite delaying their morning medications by only a few hours. Of those who were hypertensive, 37% had either an elevated SBP or DBP by the current widely accepted definition (SBP ≥ 140 mm Hg or DBP ≥ 90 mm Hg) (The Joint National Committee on Prevention, 2004). Within the sample, 39% of women and 32% of men had elevated SBP, and 9% of women and 16% of men elevated DBP during their research visit.

Prevalence of ACE and ARB Agents within the Sample

Additional analyses were performed due to the presumed effects of angiotensin upon mood and depression (Saavedra et al., 2005; Saavedra & Benicky, 2007; Saavedra, Sanchez-Lemus, & Benicky, 2011). These analyses demonstrated that a total of 55% took an ACE (angiotensin converting enzyme inhibitor), 95% CI [45.7, 64.8], and 5% took an ARBs (angiotensin receptor blockers), 95% CI [2.1, 11.1]. Many in the sample (38%) had a history of a prior myocardial infarction for which ACE drugs are standard care for those whose renal function allows their use because ACEs reduce left ventricle remodeling, recurrent myocardial infarction and all-cause mortality (American College of Cardiology Foundation, et al., 2012). A possible reason for a lower use of ACE or angiotensin receptor blockers (ARB) in this sample is that this sample's mean eGFR was 84.23 mLs/min (95% CI, [80.9, 87.6], range 43-126 mLs/min) which is less than the normal range of less than 90 mLs/min (The Renal Association, n.d.).

National guidelines indicate that ACE and ARBs can be used in most patients with chronic renal disease; there were 7 in this sample (N = 101) with an eGFR of < 60 (range 43-60 mLs/min) that would constitute a diagnosis chronic renal disease. This small cohort of seven was in stage 3 kidney disease for which clinicians often review medications and stop nephrotoxic agents such as aspirin, non-steroidal anti-inflammatories (NSAIDs), ACEs , ARBs and other nephrotoxic drugs (The Renal Association, n.d.). Thus, findings appear as expected for an adjusted sample size of 94 subjects in whom 61 used an ACE or ARB and for whom 57% had a prior positive cardiac catheterization and 38% a prior heart attack.

Additional Analyses of Associations with Blood Pressures and Hypertension

A decision was made to additionally control for demographic variables (age, sex, race, BMI, eGFR, AST and ALT) in exploring the relationship of Vitamin D levels to measures of HTN, SBP and DBP. For these regression analyses, assumptions were assessed as previously described in detail for both multivariate, linear and logistic regresions. Thus, multicollinearity was checked by a search for influential points, or outliers, by variance inflation factors (VIFs), Cook's Distance and DfBETA. There were no VIFs greater than 10 which is a value that strongly implies that multicollinearity is likely present (Mertler & Vannatta, 2013). Cook's Distance was determined and represents the general influence of a data point within the regression equation. Cook's data points more than 1 indicate influence, and there were no values > 1. A sample size adjusted Df-BETA more than $2\sqrt{101}$ (or ≥ 0.20) indicates points that may change the regression coefficients. There were ten (10) points of influence by Df-BETAs more than 0.20 in the constant (Case 3, 10, 14, 16, 20, 32, 55, 73, 77, 82). As previously reviewed, there was no multicollinearity between Vitamin D levels and SBP or DBP because their VIFs were less than 1.5, and tolerance factors all > 0.10. Normality of the studentized deleted residuals was noted by

K-S tests for both SBP ($p \leq 0.46$) and DBP ($p \leq 0.18$) but not for HTN ($p \leq 0.004$). Boxplots showed outliers for SBP (three cases); DBP (four cases); and Vitamin D level (two cases).

The multivariate linear regression between Vitamin D and SBP, DBP and HTN ($N = 101$) when controlling for age, race, sex, BMI, AST, ALT and eGFR was significant ($p = 0.05$) for the three outcomes: SBP ($R^2 = 0.28$; $R^2_{adj} = 0.21$; $F = 4.36$; $df = 8$; $p \leq 0.001$), DBP ($R^2 = 0.18$; $R^2_{adj} = 0.11$; $F = 2.54$; $df = 8$; $p = 0.015$), and HTN ($R^2 = 0.21$; $R^2_{adj} = 0.17$; $F = 3.46$; $df = 8$; $p = 0.002$). Because multivariate and linear regressions require assumptions to be met including normality, linearity *and* no significant outliers, the multivariate linear regression between Vitamin D levels and SBP, DBP and HTN was run a second time without the 10 outliers ($N = 91$) found by DfBETAs (Case 3, 10, 14, 16, 20, 32, 55, 73, 77, 82), and then a third time without the additional 7 outlier points in the blood pressure boxplots, which represented 6 additional unique points for a sample of $N = 85$. This multivariate regression without the DfBETA outliers ($N = 91$) revealed significance between Vitamin D and the demographic factors without substantial changes in the p values for all three dependent variables: SBP ($R^2 = 0.26.$; $R^2_{adj} = 0.19$; $F = 4.10$; $df = 7$; $p = 0.001$); DBP ($R^2 = 0.19$; $R^2_{adj} = 0.12$; $F = 2.80$; $df = 7$; $p = 0.012$); and HTN ($R^2 = 0.29$; $R^2_{adj} = 0.23$; $F = 4.91$; df 8; $p = 0.001$). The third multivariate regression omitted the additional outliers found within the various boxplots: SBP (Cases 70, 93, 99); DBP (30, 70, 73, 99); and Vitamin D level (5, 39) which resulted in a sample of $N = 85$ due to these additional deleted cases. The multivariate regression omitting these 16 outliers found in the DfBETAs and boxplots ($N = 85$) resulted no substantial change for the previous p values for the $N = 91$ sample for SBP ($p < 0.001$) and HTN ($p < 0.001$). However, DBP without these 16 outliers now lacked significance ($R^2 = 0.13$; $R^2_{adj} = 0.05$; $F = 1.60$; $df = 7$; $N = 85$, $p = 0.15$).

Normality was present in the studentized residuals for both the $N = 91$ and $N = 85$ sample sizes. Assumptions were met, including for the VIFs, tolerance factors and Durbin Watson

values, for each of these reduced sample size multivariate regressions. The lack of significance in DBP found when omitting all outliers follows other literature that evidences a stronger association between Vitamin D and SBP (Judd, Raiser, Kumari, & Tangpricha, 2010; Pfeifer, Begerow, Minne, Nachtigall, & Hansen, 2001; Puglisi & McCoy, 2013; Scragg, Camargo, & Simpson, 2010). Significant results in the previous multivariate models indicated that further analyses by simple linear regression for the individual variables (SBP and DBP) were appropriate but not for HTN due to the non-normality of HTN's residuals.

Next, further regressions were performed to model the effect of Vitamin D upon SBP, DBP and HTN while controlling for age, sex, race, BMI, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and eGFR. Simple linear regressions were performed using forward selection with the demographics entered in the first block and Vitamin D levels in the second block. The model of SBP as the outcome utilizing the demographic variables and Vitamin D levels performed with the variables entered in the same manner as previously described was significant ($p = 0.008$) with race, age, eGFR and Vitamin D retained in the final model: (B = - 0.26; 95% CI [-.81, -.13]; $R^2_{adj} = 0.21$, $F = 7.66$ (4, 96), $p = 0.008$). The model of DBP as the outcome controlling for Vitamin D and the aforementioned demographic factors retained only age in the final model with (B = - 0.22, 95% CI [.46, – .04]; $R^2_{adj} = 0.12$, $F = 5.39$ (*1*, 98); $p = 0.022$); thus, when controlling for demographic factors, for each increase in 1 ng/mL of Vitamin D, the odds were that DBP decreased 0.22 mm Hg and SBP decreased 0.26 mm Hg. Tolerance and VIFs were acceptable for both the SBP and DBP models, the residuals were normal by K-S ($p = 0.18$ for DBP and $p \leq 0.46$ for SBP) and Durbin-Watson values were both acceptable at less than 2.50.

Next, a logistic regression of the whole dataset ($N = 101$) to explore the association of the seven demographic variables and Vitamin D levels with a diagnosis of HTN was performed. For

this model, a Hosmer and Lemeshow test lacked significance ($p = 0.64$) which indicates good fit to the model. This overall model retained age, race and Vitamin D and was not significant in its association with HTN: AOR 0.98; $p = 0.49$; 95% CI [.92, 1.05]. The logistic regression minus the two Vitamin D level outliers ($N = 99$) revealed a good fit by the Hosmer and Lemeshow test ($p = .80$), but Vitamin D and the demographic variables were again not significantly associated with HTN (AOR = 0.99; $p = 0.76$; 95% CI [.92, 1.06).

Assumptions for a logistic regression were met as previously described. Bivariate correlations demonstrated no correlations above the acceptable limit of 0.80 between variables (Mertler & Vannatta, 2013). Assessing for outliers by a sample size adjusted DFBetas ($2\sqrt{101}$) for a value more than 0.20, and by Cook's Distance being set at 4/(n - k - 1) which in this case is 4/(101 – 8 – 1) resulted in a Cook's cutoff of 0.043 (Tabachnick & Fidell, 2007). Within the DfBetas and Cook's Distance, there were 19 total unique outliers when adding the cases of influence for Vitamin D levels, age, race and BMI. Thus, the decision was made to run this analysis without these outliers ($N = 82$) while controlling for Vitamin D and the demographic factors. Omission of these outliers yielded similar results to the full sample with no finding of a significant association between Vitamin D and HTN when controlling for demographic variables: AOR = 0.99, $p = 0.75$, 95% CI [.91, 1.07].

A possible reason for this lack of significance is that those with HTN were more likely to be on blood pressure medications the day before the research and thus may have had better residual control 24-30 hours after their last medication than those 19 subjects without reported HTN. It is a bit of an anomaly to have CAD and no HTN. It is possible subjects with or without a diagnosis of HTN had unusual blood pressures the day of the study, or that blood pressures of those with HTN were very well controlled the day of the study. This is likely because there was

no significant difference between the SBP in those with and without HTN. Mean blood pressures of those with HTN were SBP 138.6 ± 16.5, and DBP of 80.0 ± 11.1; those without HTN had mean SBP of 126.1 ± 13.5 and DBP of 78.3 ± 6.2, and these were significantly different within a Mann Whitney *U* tests: SBP ($p = 0.003$) and DBP ($p = 0.73$).

Serum Measures of Inflammation

Because the hs-CRP was available in the primary dataset and because so many other studies have demonstrated that baseline hs-CRP levels predict risk of myocardial infarction, stroke and sudden cardiac death, the serum hs-CRP was utilized herein as a serum measure of inflammation (Ridker, 2003; Strandberg & Tilvis, 2000). In the United States, median values for hs-CRP are 2.5 mg/L for women and 1.5 mg/L for men (McCormack & Allan, 2010). There is considered to be a high risk for CAD when hs-CRP levels are > 3 mg/L (McCormack & Allan, 2010).

Overall mean hs-CRP for the sample was (3.01 mg/L; *SD* 3.53; 95% CI [2.31, 3.71]) which is just above the cut-off for normal cardiac risk. As expected, the mean hs-CRP was higher for women (3.08 mg/L; *SD* 3.17; 95% CI [1.96, 4.21]) and lower for men (2.97 mg/L; *SD* 3.71; 95% CI [2.07, 3.87]).

Debate has raged whether hs-CRP is useful in primary prevention, or if it should only be used in patients at intermediate risk of coronary events to help stratify their treatment options (Boekholdt & Kastelein, 2010). Because hs-CRP levels have been significantly associated with risk of vascular mortality and risk of CAD (Boekholdt & Kastelein, 2010), it is important to control for hs-CRP in analyses as was done in this study. One study of heart failure patients (30% of whom had cardiomyopathy due to CAD) found higher hs-CRP levels were associated with the risk of readmission and mortality in heart failure patients (Alonso-Martinez, et al., 2002). Thus, a finding of mean hs-CRP levels that were above normal would be expected in this sample of

persons with CAD because low risk of CAD is found in those with < 1 mg/L, intermediate risk in those >1 but < 3 mg/L and high risk when > 3 mg/L (McCormack & Allan, 2010). Normal hs-CRP levels are typically < 3 mg/L (McCormack & Allan, 2010). The hs-CRP is an approximate measure that varies widely. In fact, one author reported a large (1.2 mg/L) standard deviation within healthy subjects on repeated measures (McCormack & Allan, 2010). This means that a person deemed to have an intermediate risk of heart disease (2 mg/L hs-CRP) on a repeated measure could be over 3 mg/L which makes risk stratification with hs-CRP alone difficult (McCormack & Allan, 2010).

This sample's mean hs-CRP was 3.01 mg/L, 95% CI [2.31, 3.71], but this mean was possibly affected by 9 outliers wherein hs-CRP levels were over 10 mg/L. Once hs-CRP is equal to or more than 10 mg/L, it is considered an acute phase reactant. Thus, levels over 10 mg/L are typically repeated in 3 weeks when the patient feels clinically well (Ridker, 2003). Of these hs-CRP outliers greater than 10 mg/L, seven were in the non-depressed and two in the depressed. Because the majority of the outliers in hs-CRP were within the non-depressed group, it is not surprising that the mean hs-CRP in the non-depressed group ($M = 3.08 \pm 3.64$) was higher than the overall mean for the sample ($M = 3.01 \pm 3/53$) and for the depressed ($M = 2.81 \pm 3.26$). However, even when outliers were removed from the full dataset ($n = 92$), overall mean hs-CRP was still higher, albeit by a smaller amount, in the groups by depression status: depressed $M = 2.00 \pm 1.91$, 95% CI [1.38, 2.62] versus non-depressed $M = 2.08 \pm 1.51$, 95% CI [1.62, 2.55]. These findings were not as anticipated because of prior literature predicting a strong association between hs-CRP and depression (Kop, et al., 2002; Raison, Capuron, & Miller, 2006; Raison & Miller, 2011).

Endothelial Measures of Inflammation

Endothelial dysfunction is present when there is ineffective or decreased vasodilatation, increased inflammation, or the endothelium displays a pro-thrombotic state (Endemann & Schiffrin, 2004). Healthy arteries vasodilate in response to hormonal stimulus, drugs, and reactive hyperemia (flow-mediated dilation) (Flammer et al., 2012). Loss of endothelium-dependent vasodilation is a typical feature present in the development of atherosclerosis and is related to future cardiovascular risk, and thus, CAD (Gonzalez & Selwyn, 2003). Due to these factors, it is not surprising that there would be an alteration of endothelial measures for persons with CAD. Endothelial measures performed herein included an augmentation index (AI) and reactive hyperemia index (RHI), both of which were measured by an EndoPAT (Itamar Medical), as well as a BAFMD which was obtained by ultrasound. It is important to understand the factors that may enhance endothelial dysfunction in CAD because having persistent endothelial dysfunction can herald worsening of CAD and poorer outcomes, this is part of the rationale for this study (Kitta et al., 2009).

Brachial artery flow mediated dilation is known to be lower in those with traditional risk factors for cardiac events (Benjamin, et al., 2004; Corretti, et al., 2002). Endothelial dysfunction is evidenced by a lesser vasodilatation of the brachial artery during BAFMD and is associated with increased age, SBP, BMI and smoking according to a secondary analysis of 2,883 Framingham Heart Study participants (Benjamin et al., 2004). The accurate measurement of BAFMD requires extensive attention to detail and equipment, and there are various unstandardized protocols for obtaining a BAFMD that makes a comparison of findings across studies difficult (Corretti, et al., 2002). These factors may preclude reliability and validity of findings in measures of BAFMD (Flammer, et al., 2012; Hamburg, et al., 2009). Thus, it is a

strength of this study that BAFMD measures were obtained by one experienced ultrasonographer (Lee, et al., 2012).

Mean BAFMD was 5.04 ± 3.45 [range – 1, 14]. A surprising finding in this study was that depressed persons had a significantly higher BAFMD compared to the non-depressed patients despite equal ranges of values: depressed BAFMD *M* = 5.62, *SD* 3.31 and non-depressed BAFMD *M* = 4.82, *SD* = 3.50. This higher BAFMD in the depressed is unexpected because BAFMD readings are considered predictive of coronary risk so one would expect lower readings in a sample of individuals with known CAD that had another condition, depression, which worsens coronary outcomes (American College of Cardiology Foundation et al., 2012; Poole, Dickens & Steptoe, 2011). Additionally, literature supports a decreased BAFMD in those who are depressed (Rajagopalan et al., 2001; Sherwood, Hinderliter, Watkins, Waugh, & Blumenthal, 2005; Wagner, Tennen, Mansoor, & Abbott, 2009).

The EndoPAT provided both AI and RHI measures. RHI is an index of suprasystolic pulse volume amplitude relative to baseline amplitude. A criticism of RHI as measured by EndoPAT is that the signal from the plethysmographic probes may be influenced by various non-endothelial factors, such as room temperature and others (Flammer, et al., 2012; Schuck et al., 2013). Another criticism is that the EndoPAT RHI index is that it is calculated by the EndoPAT software without clear indication of how the software corrects the RHI reading based on the readings from the finger of the non-occluded arm. The company's literature about the EndoPAT (Itamar Medical, n.d.) indicates that heart rate is utilized in the RHI calculation. One study found a stronger correlation between central rather than peripheral pulse pressures which means that RHI readings likely depend upon the artery utilized (McEniery et al., 2006). It is typical for persons with CAD to have a lesser RHI and BAFMD than healthy controls. In this study, RHI was induced by inflating a blood pressure cuff on the forearm 70 mm Hg above the SBP for 5

minutes (Lee et al., 2012). The RHI was found to vary by depression group: depressed M = 2.01, 95% CI [1.70, 2.31] versus non-depressed M = 2.25, 95% CI [2.10, 2.41], p = 0.05.

The science surrounding the calculation of and clinical use of endothelial measures, particularly in persons with CAD and those who are depressed, is new; this research is relatively scant and at times conflicting. The aforementioned relationship between RHI and BAFMD may indeed be due to underlying basal flow, but in this study there was a non-significant inverse relationship between RHI and BAFMD (r = - 0.08, p = 0.46) and another study indicated that pulse arterial tonometry derived RHI and BAFMD are not interchangeable measures (Allan et al., 2013).

Augmentation index is a measure of arterial stiffness based upon vascular wave reflection, but the clinical usefulness of this measure has not been established nor is it felt to be reliable (Shimizu & Kario, 2008). Arterial stiffness is thought to increase with age. The effect of AI within HTN is in debate, but AI is a measure of arterial stiffness and is typically higher in women (Fantin, et al., 2007). This increased AI in women possibly helps to explain why women as they age have a higher prevalence of HTN than men (Writing Group for the American Heart Association, 2013).

Because AI varies with sex and age and regressions were planned, the correlations between age and AI and sex and AI were calculated. In this sample there was a non-significant very small inverse relationship between age and AI (r = - 0.02, p = 0.81). In contrast, the correlation of AI and sex by a Spearman's *rho* was significant as expected: *rho* = – 0.26, p = 0.01. Mean AI, which was measured by the EndoPAT, was 23.49 (SD = 21.26; 95% CI [19.3, 27.69]), and of interest, the standard deviation was almost as large as the mean value which likely affected other analyses because of the large variability in AI (-1, 95).

In a study of non-acutely ill, non-diabetic adults of mean age 57.5 years (similar to this sample's mean age of 58), the AI of the radial artery in males was 24.9 and in females it was 30.0 (Fantin, et al., 2007). Thus, it is somewhat surprising that the current study of persons with proven CAD would have a slightly *lower* mean AI value compared to Fantin's healthy controls (18.85 for this sample of males with CAD versus 24.9 in Fantin's (2007) healthy males) (Fantin, et al., 2007) (Fantin, et al., 2007) (Fantin, et al., 2007). A finding of a lower AI in this sample of persons with CAD is unusual given that the endothelial dysfunction typical in CAD should make arteries stiffer, increasing the AI. However, this study utilized an EndoPAT with plethysmographic probes over fingertips, and Fantin et al. (2007) used PAT measures over the radial and carotid arteries by another device.

Additional Analyses of Endothelial Measures

Additional analyses were untaken to look at differences in endothelial measures by sex. Of the four inflammatory measures within this study, only AI was significantly different between the sexes ($p = 0.001$), RHI approached significance ($p = 0.07$) and BAFMD clearly did not vary significantly by sex ($p = 0.90$). As predicted, AI herein for females was quite high in this sample with CAD (33.06 ± 24.86, 95% CI [24.24, 41.87]).

Interestingly, a recent study comparing RHI and BAFMD measures in healthy controls and in individuals with peripheral arterial disease found no correlation between the RHI and BAFMD in either group or the combined sample (Allan, Delaney, Miller, & Spark, 2013). These authors concluded that pulse arterial tonometry derived RHI and BAFMD are not interchangeable measures of endothelial dysfunction which makes the non-significant findings for both measures a bit more surprising herein (Allan et al., 2013). This study did not involve healthy controls and persons with CAD to compare to the findings of Allan et al. (2013), but the correlation between

BAFMD and RHI was non-significant which lends support to their conclusion (r = - 0.08, p = 0.46).

The manner in which RHI and BAFMD were obtained does matter. In this study, the RHI reflects reactive hyperemia measured by the EndoPAT which has the advantage of measuring several vascular beds at one time and using the patient's own arm to control for and then adjust for any environmental changes. The accurate measurement of BAFMD requires extensive attention to detail and equipment, and there are various unstandardized protocols and different arteries used in flow mediated dilation making comparison of findings difficult across studies (Corretti, et al., 2002). These factors may preclude reliability of findings unless one takes care to compare studies with similar protocols to measure BAFMD (Flammer, et al., 2012; Hamburg, et al., 2009). A strength to this study is that one experienced ultrasonographer obtained all of the BAFMD readings (Lee, et al., 2012). Thus, the EndoPAT has a built-in system of controls that is not present in BAFMD which is difficult to perform reliably. These facts, along with the findings of Allen et al. (2013) help to support and explain why there were very different findings for RHI and BAFMD measures within this study. For instance, the RHI and BAFMD p values varied in significance for the depressed versus the non-depressed groups (RHI, p = 0.05 and BAFMD, p = 0.31), and for the HTN versus no HTN group (RHI, p = 0.19, BAFMD, p = 0.02). Furthermore, in the parent study from which this data was drawn, the RHI and BAFMD measured in healthy volunteers and persons with CAD (N = 139) did not vary significantly by group (Lee, et al., 2012). The authors concluded that an EndoPAT measure of RHI and an ultrasound-derived BAFMD measure may actually reflect basal blood flow (which is a function of vasoconstriction and atherosclerosis) and that vascular disease may not change the underlying physiology of the subject's digital PAT phenotype (Lee, et al., 2012).

Depression

In this study, 27% of subjects (95% CI, [19,36%]) were depressed which is a typical incidence of depression in persons with CAD (Motiwala & Wang, 2012; Pozuelo, et al., 2009). This is similar to several estimates in the literature which indicate depression in CAD is higher than in the general adult population of the United States for whom 6.7% experience depression during any 12-month time frame (National Institutes of Mental Health, n.d.). Depression prevalence has been found to be 9.3% for outpatient cardiac patients (N = 31,000) within a 12-month period (Egede, 2007). Estimates of depression's prevalence in persons with CAD vary from 15-20% of persons with various heart conditions (Pozuelo, et al., 2009), to 25-30% in persons with CAD (Rivelli & Jiang, 2007), and in bypass graft patients depression ranged from 17-44% (Khawaja, Westermeyer, Gajwani, & Feinstein, 2009). Taking a mean of the above depression prevalence estimates in persons with CAD (30% and 17-44%) yields a 30% prevalence which is in agreement with the findings of Rivelli and Jiang (2007) (Khawaja et al., 2009). This mean prevalence of depression (30%) is very close to this study's 27% prevalence and within a 95% confidence interval [19.1%, 36.1%]. Had depression been counted over the year, and not at one point in time in this sample, depression's prevalence may well have reached 30%.

The temporal relationship and causal mechanisms for depression within CAD is unclear (Poole, Dickens, & Steptoe, 2011). Some studies link platelet activation and endothelial dysfunction as potential pathophysiological links to depression, and others have commented that selective serotonin reuptake inhibitors (SSRIs), a commonly used class of antidepressants, may increase platelet reactivity (American College of Cardiology Foundation, et al., 2012; Saran, et al., 2012). Regardless of the direction of the relationship, depression poses a significant risk to persons with CAD because even when treated, there is no improvement in cardiovascular

outcomes in those with depression (American College of Cardiology Foundation, et al., 2012; Poole, et al., 2011).

Differences in Endothelial Measures in those with and without Depression

There were no significant differences in the BAFMD ($p = 0.31$) nor the AI ($p = 0.99$) in those with and without depression, but RHI did vary significantly by depression group ($p = 0.05$). Not all studies show a clear and significant relationship between endothelial measures and depression, but persistent impairment in BAFMD despite optimal therapy does appear to herald an increased risk for future cardiovascular events (Kitta, et al., 2009). In a study of stable angina patients without major psychiatric disorders who had high depression scores, subjects were found to have significantly lower BAFMD ($p < 0.001$) (Chen et al., 2013). A high depression score (which likely reflects depression), but not a higher stress score, in Chen et al. (2013) independently predicted lower BAFMD.

Consequently, it is generally believed that impaired endothelial function exists in the depressed (D. C. Cooper, et al., 2011; Sherwood, et al., 2005; Tomfohr, Murphy, Miller, & Puterman, 2011; Wagner, et al., 2009) which makes it somewhat surprising that a Mann Whitney *U* test of AI values in the depressed and non-depressed was not significant ($p = 0.99$), but the RHI was significant ($p = 0.05$). This appears to support that not all endothelial measures actually reflect the same physiological parameters.

If one considers that AI measures arterial stiffness and is dependent upon heart rate, while RHI (obtained by either BAFMD or an EndoPAT) reflect the ability to vasodilate in response to stress, it is not surprising that these endothelial parameters capture differing measures even despite the use of heart rate for the calculation of both EndoPAT derived RHI and AI. AI is an indirect measure of arterial stiffness usually calculated by augmentation pressure divided by pulse pressure x 100 (Fantin, et al., 2007). Measures of AI are associated with DBP and heart

rate making it closely correlated with cardiac function and HTN, as well as height and gender (Nurnberger, et al., 2002). Based upon this, it is not surprising that because AI was highly non-significant ($p = 0.99$) that both SBP ($p = 0.97$) and DBP ($p = 0.33$) were non-significantly different between the depressed and non-depressed. This reasoning is supported by a significant positive correlation between SBP and AI in this data ($r = 0.37$, $p \leq 0.001$) and for DBP and AI ($r = 0.23$, $p = 0.02$). Yet, it is still surprising that there was not a statistically significant difference between the depressed and non-depressed in either AI ($p = 0.99$) or BAFMD ($p = 0.31$), but that there was a slight difference in RHI ($p = 0.05$). In the parent study, RHI changes relative to baseline and BAFMD after reactive hyperemia did vary between healthy controls and persons with CAD as measured by EndoPAT but there was no relationship between the RHI by EndoPAT and BAFMD in either cohort (Lee et al., 20120). The authors concluded that RHI measures by EndoPAT and BAFMD might actually reflect basal blood flow and not be affected by an underlying digital pulsatile arterial tonometry phenotype (Lee et al., 2012).

Differences in Vitamin D Levels in those with and without Depression

The findings of no difference in Vitamin D levels by depression group was a bit surprising until one considers that because only 18% had sufficient Vitamin D levels (above 30 ng/mL), there again may not have been enough power to detect a difference by depression group because only 27 of 101 had depression. Five observational studies, including one in a cardiovascular population (May, et al., 2010), show an inverse association between Vitamin D level and depression (Ganji, et al., 2010; Hoogendijk, et al., 2008; Knippenberg, et al., 2011; May, et al., 2010; Stewart & Hirani, 2010). Additionally, two randomized controlled trials have reported conflicting results about the effect of Vitamin D supplementation upon depression (Jorde, et al., 2008; Kjaergaard, et al., 2012). Hence, it was a bit unexpected that the Pearson *r* between Vitamin D level and depression in this full sample revealed an inverse but non-

significant relationship (r = - 0.14, p = 0.17). Because the strength of the relationship between Vitamin D level and depression was small, and power for finding differences is contingent upon the effect size and alpha (herein = 0.05), the power for this question was low. Power for this query was only in the range of 11-29% which is below the traditionally sought 80% and likely resulted in an inability to find a significant inverse relationship between Vitamin D and depression (Gliner, et al., 2009).

Even when controlling for age, sex, and BMI, depression's prevalence was not affected by Vitamin D level, even with outliers excluded. Race, however, was significantly associated with Vitamin D level (r = - 0.31, p = 0.001). Because of the lack of association between most demographic variables and Vitamin D levels, it is not surprising that the regression model controlling for demographic variables was not significant (p = 0.13).

A negative correlation was expected between BMI and Vitamin D level because the extra adipose tissue sequesters the Vitamin D (Holick et al., 2011), but the correlation herein was surprisingly not significant (r = - 0.05, p = 0.61). Certainly we would expect a chronically ill sample of persons with CAD might be heavier, particularly in NC which has an obesity rate of 30% in the general population that answered the State of NC's 2012 Behavioral Risk Factor Surveillance Survey (CDC, 2013). This history of a high statewide prevalence of obesity would be expected to influence the correlation between BMI and Vitamin D level (r = - 0.05, p = 0.61), yet, this does not appear to have happened. This sample appears different from the general sample that answered the North Carolina BFSS because 55% (not 30%) were obese, and only 15 of 101 subjects had a normal BMI. Thus, the number in the sample with normal BMI may have been too low to detect a difference among variables potentially contingent upon BMI, or there may be something unique to this sample which was a cardiac sample drawn from central North Carolina which had only 33% women.

Kidney disease has been increasing in the United States in the last few decades. Older adults with kidney disease are more likely to have cardiovascular disease which makes physiologic sense because HTN frequently accompanies CAD and kidney disease. From National Health and Nutrition Examination Survey data, kidney disease in those over 80 (defined as an eGFR < 60 mL/min) increased from 41% in 1988-1994 to 51% in the 2005-2010 NHANES survey (HealthDay News for Healthier Living, 2013). Hence, another unique aspect of this sample that may have affected significance of findings between Vitamin D levels and depression or other variables, is kidney function. Vitamin D is hydroxylzed in the kidney into active Vitamin D (calcitriol) (Ross et al., 2011). A recent review citied numerous studies which demonstrate that declining renal function is associated with a lesser ability of the kidneys to produce Vitamin D (Al-Badr & Martin, 2008). It was thought, therefore, that because Vitamin D level has been significantly associated with depression, and kidney function is known to affect serum Vitamin D level, possibly eGFR might also have an association with depression. Because logistic regressions should contain all pertinent variables put in the model (Mertler & Vannatta, 2013), other analyses were conducted deemed appropriate including adding renal function (eGFR) in models with depression as the dependent variable.

Prior to performing additional analyses utilizing eGFR, assumptions were checked and met for a logistic regression to explore the effect of age, sex, race, BMI and eGFR on depression. In this model, both sex ($p = 0.045$) and eGFR ($p = 0.053$) were initially retained in the forward stepping logistic regression, but the addition of eGFR to the model did not help to establish an association with depression. Despite a reliable model (Hosmer and Lemeshow, $p = 0.20$), the final model of the demographic factors with eGFR and Vitamin D levels in association with depression was not significant (AOR = 0.97; $p = 0.25$; 95% CI [0.92 – 1.02]). This may have occurred because the high level of care provided to this sample in a well-known tertiary care

facility may have resulted in a lesser ability to detect differences in depression by eGFR because the sample's blood pressure was well controlled which may have resulted in better than usual eGFRs.

The association of hepatic function and serum Vitamin D levels was believed to be a significant potential moderator because the liver conducts a crucial step of hydroxylation prior to the formation of calcitriol (Putz-Bankuti, et al., 2012). Some research has shown that Vitamin D level may also be associated with liver dysfunction, may predict future hepatic de-compensation, and that inflammation which is associated with metabolic syndrome is also associated with elevated liver enzymes (Browning et al., 2008). In this data, the mean differences of ALT (p = 0.23) and AST (p = 0.28) were not associated with diabetes. However, mean differences between AST and ALT by depression group were highly significant with all p values ≤ 0.001. Thus, a logistic regression was also run with demographic variables in Block 1, and AST and ALT in forward stepping in Block 2 to test their association with depression. While the model was significant, only gender was retained in the model (AOR = 0.40; p = 0.049; 95% CI [.16, .99]).

Differences in Serum Blood Pressures in those with and without Depression

Blood pressures, both SBP and DBP, were not significantly different in the depressed and non-depressed (SBP, p = 0.97; DBP, p = 0.33). Notably only four subjects of the 27 with depression did not also have HTN in this sample of 101. In this dataset, the relationship between depression and HTN was non-significant (p = 0.54). Approximately equal percentages of the depressed and non-depressed had HTN: 80% in the non-depressed group and 85% in the depressed group. This overall prevalence of 80% with HTN is typical for a sample of CAD patients (Zeina, et al., 2009). It is consequently not surprising that because the prevalence of HTN was so high and so similar in the depressed and non-depressed groups, there may not have

been enough power to detect a difference by HTN as the non-significant finding suggest (p = 0.54).

Theories of why depression and HTN may be related have been proposed and evidence is beginning to accumulate of a relationship between HTN and depression but this evidence of a relationship remains scarce to date (Meng, et al., 2012; Scalco, et al., 2005). Thus, it is not surprising given the limited evidence thus far of a relationship between HTN and depression, and the volatility of HTN from moment to moment and lability of mood and depression, that the occurrence of HTN by depression group was not significant (p = 0.54).

Previous published literature has been discordant regarding the relationship of Vitamin D levels to the risk of HTN**,** SBP and DBP. Data analysis revealed in a multivariate model that Vitamin D level was significantly related to SBP and DBP (p = 0.004). There have been multiple secondary analyses of Vitamin D levels from National Health and Nutrition Examination Surveys (NHANES) data which all found an inverse relationship between serum Vitamin D level and blood pressure (A. Fraser, et al., 2010; A. K. Gupta, et al., 2011; R. Scragg, et al., 2010), but only a few randomized controlled trials of Vitamin D supplementation exist with conflicting findings (Jorde, et al., 2010; Judd, et al., 2010; Pan, et al., 1993; Pfeifer, et al., 2001). One secondary analysis of NHANEs data (N= 3,958) found that Vitamin D levels were inversely associated with SBP ($p < 0.001$) but not significantly with DBP (p = 0.19) (A. Fraser, et al., 2010; Rajakumar et al., 2011). Thus, the direction of the relationship between Vitamin D levels and blood pressure has remained inconsistent, with more studies showing an association with SBP. Vitamin D receptors (VDRs) are situated within vascular smooth muscle, and because Vitamin D is associated with muscle strength (Gupta et al., 2010; Muir & Montero-Odasso, 2011; Sinchuk & Holick, 2007; Zhu, Austin, Devine, Bruce, & Prince, 2010), one intuitively would surmise that Vitamin D levels would be more closely related to SBP which reflects the force of

contraction against the blood vessel wall. However, this association between SBP and Vitamin D has not been consistently found. Three studies did find more of a relation to SBP (Pfeifer, et al., 2001; R. Scragg, et al., 1995; Wu, et al., 2010) and one study with no relation to DBP (Wu, et al., 2010); however, a recent meta-analysis of 14 studies found no relationship between Vitamin D levels and either SBP ($p = 0.95$) or DBP ($p = 0.33$) (Elamin, et al., 2011). Further complicating the clarity of any relationship between Vitamin D and blood pressures (or other variables, too) are the following: (a) fluctuations in normal and abnormal Vitamin D levels between the earlier and later NHANEs trials, (b) use of different serum Vitamin D tests (Vitamin D^2 and D^3 or just Vitamin D^3), (c) supplementation interventions that contain or do not contain calcium (Judd et al., 2010; Pan et al., 1993; Pfeiffer et al., 2001; Scragg et al., 1995), (d) differing supplementation doses and trial lengths within the few randomized controlled trials (Judd et al., 2010; Pfeiffer et al., 2001; Ross et al., 2011), and (e) varying outcomes (SBP, DBP, HTN or normotension) utilized as the endpoint (A. Fraser, et al., 2010; A. K. Gupta, et al., 2011; R. Scragg, et al., 2010).

For these many reasons, it was unclear what this data would reveal, especially because the three NHANEs studies previously mentioned used data from healthy adults, and this trial involved a sample of persons with CAD. While findings did not reveal a significant association between Vitamin D level and HTN, there were significant associations by simple linear regression between Vitamin and SBP and Vitamin D and DBP. The adjusted odds ratio followed the weight of evidence in the literature with SBP (- 0.32 mm Hg) having a larger association with Vitamin D than that of DBP (- 0.25 mm Hg).

The surprising finding from this research question was that the logistic regression associating Vitamin D levels with HTN was not significant (Adjusted OR 0.97; $p = 0.28$; 95% CI, [.92 – 1.02]). However, power for detecting differences is always diminished when a variable is binary, such as HTN (yes/no).

Effect size measures the strength of the relationship between the independent and dependent variables (Gliner, et al., 2009). A small effect size of 0.1 is detectable by a sample of 80, and this sample is larger with 101 subjects who should allow for finding even smaller effect sizes which raises the question of how large the effect might be between Vitamin D levels and HTN. In one study, 50% of the risk of HTN in Blacks was attributed to Vitamin D levels (R. Scragg, Sowers, & Bell, 2007), and another study found that 25% of the ethnic differences in SBP were due to Vitamin D levels (Fiscella, Winters, Tancredi, & Franks, 2011). A recent trial from the Netherlands tested the hypothesis that the risk of HTN in South Asian Surinamese was partially explained by their Vitamin D levels and found that 14% of the SBP and 6% of the DBP was explained by the Vitamin D levels (Kohli et al., 2012). Hence, when detecting a small effect size a large sample is needed. In this sample of 101, 82% had HTN; therefore, it may have been difficult to detect the effect of Vitamin D levels upon the few persons without HTN. For example, when HTN is present in 75%, a sample of 100 subjects yields power of 80% (Gliner, et al., 2009) but likely considerably less power for a yes/no outcome variable such as HTN.

One rationale for the non-significant associations between Vitamin D levels and HTN and within other analyses is that data was collected over all seasons of the year for this trial, and season was not controlled for in these analyses. Results in this and previous studies might be found to vary due to differences in the samples, the season of the year, and/or the latitude/geography. Thus the current findings revealed a very small significant effect of Vitamin D for both SBP and DBP (SBP about 0.5 mm Hg decrease and DBP 0.28 in blood pressure for each increased 1 ng/mL of Vitamin D), but further studies should be done in various populations controlling for season and other known confounders of Vitamin D levels.

The Association of Measures of Inflammation with Depression

The multivariate model of Vitamin D's association with the four measures of inflammation was significant ($p < 0.001$), as were the simple linear regressions for just two of the inflammation measures: AI and the log hs-CRP. As previously stated, 82% of the sample had low or insufficient Vitamin D levels (Holick, et al., 2011) and this may have lowered statistical power to detect group differences.

An elevated hs-CRP is often higher in the depressed (Ford & Erlinger, 2004; Kop, et al., 2002; Raison, et al., 2006). Younger subjects have AI associated with both cholesterol and hs-CRP, so it is also not surprising that hs-CRP was highly non-significant ($p = 0.80$) by depression group because the AI was non-significant also by depression group ($p = 0.99$) (Writing Group for the American Heart Association, 2013).

Data suggests that systemic inflammation (including hs-CRP) has been significantly associated with liver function, including ALT (Browning, et al., 2008). The results from this dataset did not show significant associations between hs-CRP and either AST or ALT. This data likely varied from the findings of Browning et al. (2008) because this data set involves only persons with CAD (24% of whom were diabetic and 23% of whom smoked), and Browning's study utilized only overweight females that were not diabetic and allowed light smoking. Thus because of prior research linking AST and ALT to a state of inflammation, a multiple linear regression of ALT, AST and Vitamin D levels was conducted. The multiple linear regression of ALT and AST's effects upon Vitamin D level met assumptions, but it was not significant ($R^{2\,adj} = 0.019$; $df = 98$; $p = 0.38$). Another regression was performed due to known associations between hs-CRP and depression and the potential association between ALT and hs-CRP (Browning et al., 2008) with depression as the dependent variable. This logistic regression utilized the demographic variables (age, sex, race, BMI), hs-CRP (Block 2) and ALT and Vitamin D level

(Block 3). This overall model was significant (AOR 0.40; $p = 0.049$; 95% CI [0.16, 0.99]) but only sex remained in the model in association with depression, and sex is a variable known to be highly related to depression prevalence with women having approximately twice the rate of depression (Schneibe, Preuschhof, Cristi, & Bagby, 2003).

Additional Analyses for Season and Other Factors affecting Vitamin D Levels

Vitamin D is obtained by supplementation, food sources, or by sunlight exposure. Consequently, the effect of season upon Vitamin D level and dependent variables that may be affected by Vitamin D warrants close examination. Vitamin D levels vary significantly by season, and this was demonstrated by the significant correlation found herein with colder seasons being associated with a lower Vitamin D level ($r = 0.35$, $p \leq 0.001$) (Holick, 2004; Holick, et al., 2011). The development of a reliable laboratory correction factor for the season of Vitamin D analysis, and the re-analysis of prior studies based upon season of Vitamin D level analysis, might lead to more stable and reliable findings with regards to the effects of Vitamin D level upon depression, blood pressures and inflammation. Thus, in order to more accurately assess the Puglisi model, future studies should control for factors that may potentially affect Vitamin D levels and/or depression: (a) season and latitude, (b) sunscreen usage, (c) clothing choice, (d) amount of time spent outdoors weekly, (e) darkness of skin pigmentation, (f) kidney and liver disease (sites of Vitamin D hydroxylation), (g) malabsorption syndromes (because Vitamin D is fat soluble), (h) medications that may result in an increased need for Vitamin D intake, and (i) the amount and frequency of exercise (Ross et al., 2011). Of interest, season and race (Black and non-Black in this study) were able to predict 18% of the variance in Vitamin D levels, and these factors should be controlled for in future studies ($R^2 = 0.20$; $R^2_{adj} = 0.18$, *df 2;* $F = 12.04$, $p \leq 0.001$). This may provide further evidence to whether there is a link between Vitamin D, blood pressure, inflammation and depression. For instance, one study undertaken to assess the effect of

depression on HTN found that depression is an independent risk factor for HTN (Adjusted RR 1.42, 95% CI [1.09, 1.86], $p = 0.009$) (Meng et al., 2012). In this study 85% (23 of 27) of those with depression had HTN.

Diet has been shown to be a confounder that might affect Vitamin D levels in this and other studies. A recent study of the effects of season and diet upon the Vitamin D status of White and Black children revealed that race, season and diet were predictors of serum Vitamin D levels; however, dietary intake of Vitamin D only assisted in supporting the serum Vitamin D levels of White children in the winter (Rajakumar, et al., 2011). There were only 19 African Americans in this study, so this may have decreased the ability to detect differences due to the small number of the sample that were of non-White race. Surprisingly, in another study the Vitamin D levels of White and Black children in Pennsylvania did not vary significantly by sufficiency or insufficiency, but did by race and season which reliably predicted the serum Vitamin D level (Rajakumar, et al., 2011). Hence, an additional multiple linear regression was conducted after assumptions were assured. This analysis showed a significant relationship between race (Black and non-Black), season and serum Vitamin D levels ($R^2 = 0.20$; $R^2_{adj} = 0.18$; *df 2;* $F = 12.04$; $p \leq 0.001$) which concurred with the previous finding of Rajakumar et al. (2011).

Vitamin D levels are also affected by demographic factors. Skin pigmentation, which is determined by the melanin content within skin, is capable of reducing cholecalciferol synthesis in response to exposure to ultraviolet-B light (Glass, Lens, Swaminathan, Spector, & Bataille, 2009; Hall et al., 2010). Of this sample, 19% were Black (which is less than the state average of 22% stated earlier) and Blacks typically have darker skin pigmentation than Whites. Vitamin D levels varied significantly with race in this study ($r = - 0.31$, $p = 0.001$). Whites had a mean Vitamin D level of 22.4 (95% CI [20.4, 24.4]) and Blacks a mean of 15.1 (95% CI [11.5, 18.8]). The mean

values were significantly different by race ($p \leq 0.001$), as others have shown (Fiscella, 2010; Puglisi & McCoy, 2013).

Season of the year, as well as sun exposure, affects serum Vitamin D levels. A third of participants (33 of 101) had their serum drawn between November and February when dermal conversion is decreased by 80-100% (Holick, 2010). Another 30% of the sample had their serum drawn between March and May and the remaining 33% had Vitamin D levels drawn during the peak season. There is no known correction factor published for adjusting serum Vitamin D levels by season of the year, but likely seasonality, and not merely physical illness, affected some of the findings in this study and perhaps many of the other studies on Vitamin D levels published to date. This lack of adjustment for seasonality for Vitamin D levels might account for the decried divergent results found in many studies (Zitterman, Borgermann, Gummert, & Pilz, 2012). Further evidence of the need for a correction factor for serum Vitamin D levels by season in this research and other studies is supported by the statistical differences in the Vitamin D levels per season. Levels are significantly lower herein between November and February versus June through October. Vitamin D level and season within this dataset were significantly related ($r = 0.35$, $p < 0.001$).

Exploring the Puglisi Model of Vitamin D Levels' Associations with Depression

None of the final three exploratory research questions yielded significant models. The demographic factors, Vitamin D levels, blood pressures nor measures of inflammation significantly influenced depression in the exploratory research questions in this sample of persons with CAD. For instance, the multiple regression answering 3-RQ4 utilized forward selection, and in the first and second iterations all three HTN, and then all four inflammation variables, were removed. Thus, it is not surprising this model was not significant ($p = 0.66$). As an additional analysis, the order of Block 1 and 2 were reversed with inflammation measures entered into

Block 1 by the forward Wald method and HTN measures in Block 2 by forward Wald method. Results again lacked significance ($p = 0.33$). This is unexpected given that the individual models 3-RQ1 and 3-RQ2 both blood pressures and inflammation were associated with Vitamin D level, and has been previously associated with depression (Ganji, et al., 2010; May, et al., 2010). Thus, a decision was made to assess the correlations of HTN, SBP, DBP, hs-CRP, BAFMD, RHI, and AI with depression and none were found to be significant with p values ranging from a low of $p = 0.12$ for RHI to $p = 0.73$ for hs-CRP which was not a normally distributed variable.

Another possible explanation for non-significant findings between these final three exploratory questions is that the relationship between depression and CAD may be very complex. Prior literature reflects a lower incidence of depression in those more than age 60, but this did not hold true for this sample which was different than a community sample because the sample was more chronically ill and older (Kessler, et al., 2005). This sample had 31% depressed that were over age 60, but only 23% of those under age 60 depressed, and this atypical finding may account for some of the unexpected findings within the questions for which depression was an independent variable. Another possible explanation for the lack of significant results is that depression, while prevalent at an increased rate in CAD over the general population (27% in this study and 15-44% in others versus 6.7% for the general population) may be only marginally affected by each of these factors such that when put together into regressions they yield non-significance (Khawaja et al., 2009; National Institutes of Mental Health, n.d.; Rivelli & Jiang, 2007).

The Puglisi model does not depict directional associations between inflammation and blood pressure with depression. One explanation for blood pressure to possibly have a very small effect on depression is that 82% of the sample was on beta blockers which have been historically thought to induce depression. However, this presumed effect of beta blockers was recently

discounted by an analysis that suggested beta blockers do not induce depression (Muzyk & Gagliardi, 2010). Perhaps a more significant reason for the lack of significant findings in these exploratory questions with depression as the outcome is that 55% of subjects were on an ACE. Some evidence suggests that ACEs may be mood enhancing, anxiolytic or may actually decrease brain inflammation which would have made finding significance in this cardiovascular sample more difficult in Specific Aim 3, questions two through five because each of these questions involved depression as the outcome (Saavedra, 2012; Saavedra, et al., 2011).

A possible explanation for there to be a lack of significant findings of an association between Vitamin D levels and some measures of inflammation is that the vast majority of this sample were on two drugs that decrease inflammation: 97% were on aspirin therapy and 93% on lipid lowering (statin) drugs (Antonopoulos, Margaritis, Lee, Channon, & Antoniades, 2012). The use of medications that decrease inflammation by this sample of primarily men (66%) with CAD might make detecting differences more challenging; women are known to have higher levels of hs-CRP and some other markers of inflammation so there may not have been enough of them in the sample to find significant differences. Consistent NSAID use may increase cardiovascular risk by impairing platelet function and by negating the cardio-protective properties of prostacyclin which relaxes blood vessels and unglues platelets (Yua et al, 2012). Because this was a sample that was of mean age 58 years, there is a possibility some may have been taking unreported over-the-counter NSAIDs which they may have neglected to report, thinking as many patients do that non-prescribed and as-needed over-the-counter medications are not reported because they are not conceived of as "medications" by laypersons. Such a factor would have likely decreased the subject's hs-CRP levels and possibly altered their endothelial measures of inflammation. Beyond their possible suppression of hs-CRP values, the chronic use of NSAIDS, as the withdrawal of Rofecoxib nearly a decade ago showed (Medicine and Healthcare

Regulatory Agency, 2004) would possibly increase subjects chances of being included in this study as a cardiovascular patient, and of having the attenuated BAFMD and RHI known to accompany CAD (Schuck, et al., 2013). Thus, the aforementioned factors and the substantial proportion of those deficient in Vitamin D level (52 of 101 herein) may have worked to mask any relationship(s) between Vitamin D levels and measures of inflammation.

Because demographic variables, Vitamin D, blood pressure and inflammation variables did not appear to be significantly associated with depression, the author chose to also explore the effect of renal function (eGFR) and liver function (ALT) upon depression. An additional analysis with hepatic function to examine a multivariate model of Vitamin D's relationship to hs-CRP, BAFMD, RHI, AI and ALT was significant ($p < 0.002$). Assumptions for this multivariate model with ALT added were assured. All 5 studentized deleted residuals were normal by K-S tests, there were no outliers of the normalized residuals by boxplot and the Q-Q plot of the expected versus observed residuals were essentially normal. VIF and tolerance factors were within acceptable limits, and there were no high correlations between variables. All assumptions were met, and the multivariate model with ALT again showed a significant relationship between Vitamin D level and measures of inflammation ($p \leq 0.001$).). However, when a multivariate model of Vitamin D levels relationship to hs-CRP, BAFMD, RHI, AI, ALT and eGFR was run, this model was also significant ($p = 0.004$).

Implications for Nursing

Many adults should be routinely screened for Vitamin D deficiency: those with altered renal or hepatic function, the elderly, and those with dark skin pigmentation (Holick, et al., 2011). This study found that 82% of subjects had abnormal Vitamin D levels, and 52% had a clearly deficient Vitamin D level. Nurses should ascertain if patients have risks for Vitamin D deficiency by asking if patients: ingest Vitamin D or mixed ingredient calcium supplements or cod liver oil;

have malabsorption syndromes; spend time out in the sun during mid-day; utilize sunscreen which blocks ultraviolet light; and consume the few foods that are high in Vitamin D (supplemented dairy and orange juice, salmon and/or shitake mushrooms). Nurses should also be aware that because so much of society has inadequate serum Vitamin D levels, the recommended daily allowance has been raised to the current 600 IU for ages 1-70 and 800 IU of Vitamin D for those above age 70 (Ross et al., 2011a).

As regards depression, nurse practitioners (NPs) and other clinicians should also be aware that SSRIs have been heavily used in persons with CAD because they were deemed safe in that population (Glassman et al., 2002; Lesperance et al., 2007). However, SSRIs do hold the risks of increased platelet aggregation which may explain why even when depression is identified, treated and controlled, cardiovascular outcomes do not improve (Writing Committee for the ENRICHD Investigators, 2003**)**. Thus, SSRIs may actually be a heavily studied agent in cardiac patients, but they may not be the best agent in persons with CAD, especially those who have other risks for thrombosis or prior thrombotic events.

This study clarifies why in accordance with the American Heart Association guidelines, nurses and other healthcare providers should screen persons with CAD for depression when appropriate supports are in place to initiate treatment (American College of Cardiology Foundation, et al., 2012). The current Endocrine Society guidelines do not encourage supplementation with Vitamin D to decrease the occurrence nor the risk of cardiovascular disease, depression, or to improve quality of life (Holick, et al., 2011). Perhaps well-designed future research with careful controls for factors that influence Vitamin D levels (age, BMI, season, sunscreen use, latitude, sun-seeking behaviors, and supplement usage).

Healthcare providers examining persons with CAD should be aware that 82% of persons with CAD have HTN, and depression, when present and regardless of whether it is controlled or

not, increases morbidity and mortality (American College of Cardiology Foundation, et al., 2012; Olafiranye et al., 2011). Vitamin D levels have been found observationally in multiple secondary analyses to be associated with decreased SBP, and at times, DBP (Forman, 2007; Fraser, 2010; Judd, 2010; Pfeifer et al., 2001; Scragg et al., 2010; Swales & Wang, 2010). Randomized controlled trials show mixed findings to date of Vitamin D's association with blood pressure (Judd, et al., 2010; Pan, et al., 1993; Pfeifer, et al., 2001; M.D. Witham, Nadir, & Struthers, 2009; Miles D. Witham et al., 2013); thus, supplementation is not yet the supported in order to reduce blood pressure (Holick, et al., 2011). Yet, this study showed that Vitamin D levels were significantly related to SBP and DBP. Thus, because low Vitamin D levels lead to more adverse events in cardiac populations, it is reasonable for astute clinicians to consider screening patients deemed at risk of hypovitaminosis D, including HTN patients (who may have early, silent CAD) and patients with known CAD, and treating patients accordingly (Zitterman, et al., 2012).

Because endothelial measures are becoming more commonly utilized, and the tests are becoming more reliable and standardized, with clinical implications for establishing early disease and identifying when there is an increased risk of events, their use allows clinicians the opportunity to potentially reduce adverse cardiac events. Thus, as the tests begin to be utilized to guide diagnosis and treatment in primary and secondary prevention, it is important for clinicians to understand what the tests are and how results vary in the setting of CAD and which factors alter the results of endothelial function. In this cardiovascular sample, endothelial measures within the complex exploratory aims were found to not affect depression (Corretti et al., 2002; Deanfield, Halcox, & Rabelink, 2007; Meirelles, Leite, Montenegro & Gomes, 2007; Sherwood et al., 2005). However, AI and hs-CRP were significantly related to inflammation, and elevated hs-CRP and other cytokines are often increased in those who are fatigued or depressed, although hs-CRP and depression were not significantly correlated herein (r = - 0.034, p = 0.734) (Kop, et

al., 2002; Raison, et al., 2006; Raison & Miller, 2011). Persistent impairment of endothelial function in persons with CAD by serial measurement over time was initially thought to be associated with adverse outcomes (Kitta, et al., 2009). However, recently a comparison of BAFMD and RHI results in healthy individuals and persons with CAD found that the results may be due to underlying basal flow conditions and not due to the presence of vascular disease clearly altering vessel response (Lee, et al., 2012).

Based upon emerging evidence, nurse practitioners (NPs) should consider that patients who are hypertensive or depressed, particularly when they have known risk factors for low Vitamin D levels, should be screened for low serum Vitamin D and depression when they are at high risk for or have established CAD. Even for patients rarely outdoors, seasonal variations may occur because it is now recognized that the altered solar zenith angle in winter produces an 80% reduction in Vitamin D synthesis even in Florida (Holick, 2010). Thus, NPs may elect to screen patients at risk of low Vitamin D in the late fall or winter when Vitamin D levels are at their nadir in order to intervene effectively to improve outcomes. It remains unclear if Vitamin D supplementation would help patients lower their blood pressure or improve their mood because evidence to date remains conflicting. The Endocrine Society does not suggest that supplementation might decrease occurrence or risk of cardiovascular disease, depression, or improve quality of life (Holick, et al., 2011), but others have started to suggest that Vitamin D supplementation may be a low-cost therapeutic needed by many and which may be useful in improving blood pressures.

Future Directions of Research

Research can identify variables of importance, clarify relationships of the variables, and allow scientists to theorize about and discover new relationships which then guide future inquiries. The research process presents opportunities and frustrations, but nursing research holds

the promise of making progress of creating theories that lead to findings that are capable of improving the patient's health and environment. This study was performed on a cardiovascular population as guided by a nurse's physiologic theory that proposed a relationship between depression, blood pressure, serum and endothelial measures of inflammation and Vitamin D levels. Hence, results can only be generalized to another cardiovascular population. Most cardiac patients have a reduced exercise capacity, so it is recommended that future research of depression and Vitamin D levels in cardiac patients should include their functional capacity and physical activity level to see if these significantly affect their Vitamin D levels and depression (Zitterman, et al., 2012) because certainly one would expect a more active patient might have more sun exposure, and better blood pressure control and likely better endothelial function.

Several theories of depression might explain a relationship between depression and HTN: (a) increased stress results in cortisol or catecholamine secretion which increases heart rate and blood pressure (Fraser et al., 1999); (b) the smaller pre-frontal cortex found in those with depression which limits executive function (Fields, 2012) might also be associated with other brain or cardiac abnormalities that might contribute to HTN, and, (c) plasticity of the blood-brain barrier might allow any increased cytokine release or tumor necrosis factor to stimulate the vagus nerve and increase sympathetic activity (Tracey, 2002). Because some plausible theories support a relationship between HTN and depression, another study exploring their association, as well as the temporal nature of their relationship (did the depression lead to HTN or the other way around) would be useful. Such a study would test the relationship between blood pressures and depression proposed in the Puglisi model, and provides a direction to the relationship between these two chronic conditions.

There were notable limitations for this study. Blood pressures were measured once manually and without inter-rater reliabilities assessed. It may be that because of questionable

reliability of the blood pressures that Vitamin D levels were found to be significantly related to blood pressures (SBP and DBP), but not the diagnosis of HTN. The secondary analysis of a cross sectional design was not optimal, but certainly had advantages of expediency, low cost and an availability of equipment and expertise by other research personnel that the PI could not have afforded to utilize for a dissertation. A random sample with multiple measures of inflammation, blood pressures, depression and serum (hs-CRP, Vitamin D, liver and renal function) over time would have strengthened the design flaws of a convenience sample with one cross-sectional measure in a study set up to answer questions other than what this researcher chose. Another consideration that may limit accuracy of findings is that it is not known if the prevalence of depression would be different for persons recently diagnosed with CAD, during an acute cardiac event, or when hospitalized as an inpatient treatment as compared to this sample's stable outpatient CAD subjects. This raises the pertinent point that the diagnosis of depression was recorded from the record when the patient was recruited in the catheterization lab, which means that the 27% of the sample of persons with CAD in this sample arrived at the catheterization lab with a current, established diagnosis of depression, but there may have been undetected depression, because patients were not assessed with any tool for depression.

Some strengths of this study bear mentioning. First, clinic visits for participants were widely dispersed in the seasons with about a third occurring in November-February, March-May, and June through October. Thus, Vitamin D levels were drawn in approximately equal proportions throughout the year. As previously mentioned, one sole ultrasonographer collected all the BAFMD levels which likely would increase the reliability of the procedure and validity of results. The EndoPAT (Itamar Medical), used for the AI and RHI readings, is a Food and Drug Administration approved device for collecting endothelial measures and estimating the health of

the vasculature and the subjects serve as their own controls with the EndoPAT correcting for any systemic changes during the course of the test.

It was surprising in this study that Vitamin D level was not significantly associated with depression given that previous observational studies of Vitamin D levels' association with depression, and RCTs involving supplementation of Vitamin D, both found an effect of Vitamin D as an intervention or Vitamin D levels upon depression (Ganji, Milone, Cody, McCarty, & Wang, 2010; Jorde, Sneve, Figenschau, Svartberg, & Waterloos, 2008; May et al., 2010; Stewart & Hirani, 2010). Of these studies, only May et al. (2010) was specifically a cardiovascular population; consequently, Vitamin D may act differently within cardiovascular sample. Perhaps given that Vitamin D receptors are in the cardiomyocytes, the vasculature and the brain (Bertrone-Johnson, 2009), there is some preferential or more robust binding at various sites or in the state of deficiency that makes Vitamin D exert different physiologic effects depending on the severity of the deficiency, number and availability of binding sites and other factors yet not known. Thus, recommendations for future research would include screening for depression with a commonly used reliable and valid tool, such as a Patient Health Questionnaire 9 (PHQ-9) or Beck Depression Inventory tool. Such tools would help the PI to verify current ongoing depression despite treatment versus new depression that was yet to be diagnosed. Also, because depression is a labile mood state, measuring mood twice perhaps a week or two apart would be a more useful indicator of mood stability to assess if individuals with persistently lower depression scores would perhaps have more inflammation, higher blood pressures and lower Vitamin D. Test-retest reliability of the PHQ-9 in a primary care sample at 48 hours was 0.84 (Kroenke et al., 2001) which is acceptable, but at one week another sample had a Cronbach's alpha of 0.59, which is less than the usual acceptable Cronbach's alpha of 0.80 but which might be expected when assessing a mood state (Monahan et al., 2007). Thus, repeated measures of depression screening

with a reliable tool, as well as repeated lab work and endothelial measures would perhaps paint a more telling story, especially given that consistently elevated, not randomly elevated one-time endothelial measures, paints a more foreboding picture for cardiovascular outcomes (Kitta, et al., 2009).

The exploratory questions (Specific Aim 3, questions 3-RQ3-3RQ5) did not clearly support the Puglisi Model's premise of the indirect association between inflammation, Vitamin D and depression. Like all other studies, however, there were limitations. The sample utilized herein was a convenience sample, and because of the suspected acute symptoms causing some subjects to be sent to the catheterization lab at UNC Hospitals, it is possible some important medical diagnoses (i.e., depression), or medications (anti-depressants), may have been initially not recorded in their medical record, resulting in the omission of a current clinical diagnosis of depression. Additionally, subjects may have failed to disclose their history of depression fearing a negative judgment by the health professionals providing their care during a frightening acute pain episode. Also, this study counted a diagnosis of depression in the medical record, but just because a person has a prior diagnosis of depression does not mean that they currently have depression; in other words, the subject's depression may have resolved.

In the exploratory questions (Specific Aim 3, Questions 3-5), neither blood pressure measures nor any measure of inflammation (BAFMD, RHI, AI, hs-CRP) was associated with depression despite prior research, some of it with samples of older adults or cardiovascular samples like this one. This research suggests there may be a relationship between Vitamin D levels, blood pressure and inflammatory measures upon depression, but that something may be unique to cardiovascular patients with CAD (Kop et al., 2002; Sherwood et al., 2005). Certainly, it is clear that cardiovascular patients with depression, even when treated, have worse outcomes, so it may behoove future researchers to cast a wider net to find a yet undiscovered variable that

may be a key driver in the association of depression in those with CAD. Thus, further research is needed to clarify if serum or endothelial markers of inflammation are indeed different in healthy versus cardiovascular cohorts, and if they are indeed significantly associated with depression which would give support to the proposed Puglisi model. Testing the Puglisi model in a sample of healthy adults and in persons with CAD to see if differences exist in Vitamin D level, blood pressure and inflammatory measures may lay the groundwork for understanding how the relationship between these variables differs in persons with CAD. Additionally, research exploring the differences between depression and either vital exhaustion or sickness behavior with their associations with inflammation and Vitamin D would be useful because of the overlap in the conceptual domains. Discovering the key drivers between these variables will likely allow tailored treatments to be developed that have the potential to improve outcomes in persons with CAD, especially those with CAD and depression, in order to decrease the personal, financial and societal costs of CAD.

Summary

The purpose of this study was to examine the association of demographic factors, serum Vitamin D levels, blood pressure measures, and serum and endothelial measures of inflammation upon the prevalence of depression in persons with CAD from central North Carolina using the Puglisi model as the guiding conceptual model. Within this sample of persons with CAD, expected numbers had HTN (81%) and (27%) depression, and most were deficient in their serum Vitamin D levels. Despite prior research suggesting a relationship between Vitamin D levels and depression, no such relationship was found in this sample of 101, although there were expected relationships between Vitamin D levels and age, race, SBP and DBP, and hs-CRP. Vitamin D levels were not significantly related to BAFMD nor RHI.

In fact, hs-CRP's association with depression was not significant despite prior research. The endothelial measures as a group were not significantly associated in exploratory models with depression as the outcome, although mean RHI differed in those depressed and not depressed. Of interest, neither RHI, nor BAFMD, correlated significantly with each other as noted in prior research which suggests that BAFMD and RHI may measure different vascular effects. Thus, in this study, BAFMD was surprisingly not significantly associated with depression despite prior research supporting this in young adults, post-menopausal women with diabetes and in those with coronary artery disease (Rajagopalan, et al., 2001; Sherwood, et al., 2005; Wagner, et al., 2009).

When testing the Puglisi model in the three exploratory questions (Specific Aim 3, RQ 3-RQ5), there was no significant association between blood pressure measures and serum and endothelial measures of inflammation with depression. Thus, future research should compare persons with CAD to healthy control for factors that are thought to affect Vitamin D levels and mood. Furthermore, future research should include a sample of persons with CAD and healthy controls utilizing a widely recognized depression tool (such as the Patient Health Questionnaire 9 or the Beck Depression Inventory) along with rigorous measurement of serum and endothelial markers at multiple points in time. Such an approach would allow a comparison of healthy and CAD cohorts to determine if the Puglisi model explains more in one population or another. Examining data over time would allow a better understanding of the effect of stability of measures of depression, blood pressure, serum and endothelial markers of inflammation. Finally, seasonally adjusted Vitamin D levels followed over time would assist in determining their association with depression, and if findings differ in various populations.

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